

## Declaration

I, Kuniaki SHIMIZU, a national of Japan, c/o Asamura Patent Office of 331-340, New Ohtemachi Building, 2-1, Ohtemachi-2-chome, Chiyoda-ku, Tokyo, Japan do hereby solemnly and sincerely declare:-

- 1) THAT I am well acquainted with the Japanese language and English language, and
- 2) THAT the attached is a full, true, accurate and faithful translation into the English language made by me of Japanese Text of the U.S. Serial No. 09/678,652 filed on October 4, 2000.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001, of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 12th day of December, 2000.

Kuniki Shimizu

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Patent Attorney

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A METHOD OF INSPECTING A DNA CHIP AND  
AN APPARATUS THEREOF

BACKGROUND OF THE INVENTION

The present invention relates to an apparatus for reading and analyzing an emitted light (fluorescent light) pattern emitted from a sample located on a  
5 plane. More particularly, it relates to a method and an apparatus by which living body samples, such as a DNA, a RNA and an oligonucleotide marked by a fluorescent material, are trapped at a plurality of positions on a substrate so as to read an emitted light  
10 pattern from the fluorescent marker with a high-resolution, at a high-speed and with a high-sensitivity.

Analysis technologies for analyzing the DNA and proteins are important in fields of medicine and  
15 biology including gene analysis and gene diagnosis. In particular, in recent years, attention has been focused on a method and an apparatus by which, using a DNA probe array (which is called in other way by various names such as oligochip, DNA chip or biochip, but here-  
20 inafter the DNA probe array is employed as the generic name), a variety types of DNA sequence information and gene information are inspected and analyzed simultaneously from one specimen to be inspected. The DNA probe array is implemented in the following way: A substrate

such as a glass is used and separated into a plurality of (millions to tens of millions) areas, and target (usually, different types of) DNA probes are fixed onto the respective areas so as to form the respective areas into microscopic reaction areas, thus implementing the array. The reaction of the specimen with the array allows a target DNA in the specimen to be trapped in a state of being hybridized with the fixed DNA probes. Moreover, the target DNA is coupled with a probe such as a fluorescent probe, thereby making it possible to measure the coupled state (the position, i.e., the hybridized sequence) and its quantity with the use of the fluorescence intensity and so on. This permits the array to be utilized in the gene diagnosis and the sequence.

A microscope-like apparatus (confocal fluorescent microscope), which is usually called a scanner, is used in order to read the fluorescence intensity emitted from the fluorescent marker on the target DNA trapped in the respective reaction areas of the DNA probe array (for example, refer to JP-A-11-315095). In this apparatus, the array is irradiated with a excitation light such as a laser light which has been modified into one microscopic spot-shaped configuration, and fluorescent lights generated are separated from the excitation light using a spectroscopic device such as an interference filter, then detecting the fluorescence intensity using a photo detector such as a

photomultiplier tube. At that time, the excitation light is waved using a galvanometer mirror so as to scan, in a 2-dimensional manner, the microscopic spot formed on the array. Otherwise, the position of the  
5 microscopic spot is fixed, then scanning the array in the 2-dimensional manner. These scans make it possible to recognize the fluorescence intensity distribution of the entire array, i.e., the degree of the coupling toward each DNA probe.

10           When trying to apply the DNA inspection to a living body inspection such as a conventional blood inspection, it becomes absolutely required to execute the inspection toward a large number of living specimens at a high-speed. In the conventional  
15 technologies, however, it takes so much inspection time to irradiate one point with the excitation light spot, which is equivalent to a necessary inspecting resolution of the DNA chip, and to detect the obtained fluorescent lights one after another. This is  
20 concerned with the fact that it is impossible to shorten unlimitedly a time needed to detect this one point. Namely, this is attributed to the fact that the time  $\Delta t_L$ , which is needed up to the finishing of the fluorescent light generation after the irradiation with  
25 the excitation light, is equal to substantially 10 ns. Transferring the detection to the next detection point without waiting for the finishing of the fluorescent light generation makes the detection itself impossible.

Also, it is required to perform the detection with a high-sensitivity that corresponds up to a state where there exist several fluorescent molecules within the spot light size equivalent to the above-described  
5 necessary detecting resolution. However, all of the generated fluorescent lights are not detected. Namely, a light utilization efficiency of the detecting optical system and a quantum efficiency of the photomultiplier tube used for the optical detection are not equal to  
10 100%. Moreover, the following values are small: An efficiency with which the excitation light is absorbed in the fluorescent material and a probability with which the absorbed excitation light is converted into the fluorescent light. This requires that the detec-  
15 tion be executed by spending a time that is at least tens to hundreds times longer than  $\Delta t_L$ . Furthermore, the more this time is lengthened, the higher the accuracy of a weak light detection becomes which is almost the same as the photon counting.

20 Also, in order to accomplish such a high-speed characteristic at a practical level, the following conditions are required: Eliminating or reducing influences of foreign substances that are composed of various types of proteins mixed into the DNA chip, or  
25 maintaining all the time a focal point of the detecting system onto the inspection plane on which the fluorescent material-added target has been hybridized. Also, in some cases, it becomes necessary to perform

the detection with reference to a plurality of fluorescent lights at a high-speed.

When forming multi-spot lights from a beam emitted from a laser light-source, if the emitted light is wished to be modified into the multi-spot lights without being wasted, i.e., for example, when trying to form 50 multi-spot lights that are 0.04 mm in diameter and are arranged in a line with a pitch of 0.4 mm, the emitted laser beam is formed so that the beam becomes an ellipse-shaped configuration the longitudinal-to-transverse ratio of which is equal to 1 to 50. Irradiating, with the ellipse-shaped beam, a microlens array where microlenses are arranged with a pitch of 0.4 mm makes it possible to obtain the 50 multi-spot lights about 0.04 mm in diameter and having the pitch of 0.4 mm, if a focal length of the microlenses is equal to 60 mm, for example. Since the intensity distribution of the laser beam is Gaussian distribution, in the arrangement of the 50 multi-spot lights formed in the method as described above, spots situated in proximity to the center of the arrangement become lighter and spots situated in proximity to the periphery thereof become darker. Trying to equate as much as possible the intensities of the spots at the center with those of the spots on the periphery requires the following: A spread area of the incoming ellipse-shaped beam should be made large enough as compared with the width of the 50 microlenses. As a

In the DNA probe array, the number of the types of the fixed probes is increased and thus its density is becoming more and more heightened. On account of this, an apparatus for measuring the fluorescent marker distribution of the DNA probe array is expected to operate at the high-speed and with the high-sensitivity in addition to implementing the high-resolution characteristic. In the above-described apparatus, however, no sufficient consideration has been given to this point. Namely, it is required to make the spot diameter of the excitation light more microscopic in order to implement the high-resolution characteristic, whereas the scanning time is increased at that case (when an exposure time for each spot is identical). Moreover, making the spot diameter smaller lessens the number of the fluorescent molecules existing within the spot diameter, thereby lowering the detection sensitivity. Also, in order to implement the high-speed characteristic, it is required to increase the scanning rate or to make larger the spot diameter of the excitation light. Increasing the scanning rate, however, shortens the excitation time for the fluorescent material, thereby lessening the fluorescence intensity or increasing a circuit noise to lower the detection sensitivity. Also, in order to implement the high-sensitivity characteristic, it is required to make





efficiency is no good and the light intensities are decreased, thus resulting in such a problem that the detection sensitivity is deteriorated.

In a method of focusing a one-beam laser  
5 light onto a target to be inspected, the detection is executed on a one-pixel by one-pixel basis in a time-sequence manner. As a result, when the fluorescent light is extremely weak, it takes a time to execute the detection equivalent to the one-pixel. Consequently,  
10 it takes a considerable amount of time to detect the entire 2-dimensional image. Even if an excitation light of the one-beam laser is strengthened in order to detect the weak fluorescent light emitting target at a high-speed, when trying to detect, for example,  $6000 \times$   
15  $6000$  pixels in 1 minute, the detecting time per pixel turns out to become 1 to 2  $\mu\text{sec}$  (microseconds). Executing the detection in such a short time makes it difficult to detect the fluorescent light detection intensity in a broad dynamic range (for example,  $2^{16}$ ).  
20 Also, since a normal fluorescent material emits the fluorescent light with a delay of  $10^{-9}$  to  $10^{-5}$  sec after receiving the excitation light, the above-described per-pixel fluorescent light detecting time of 1 to 2  $\mu\text{sec}$  prevents the fluorescent light detection from  
25 being fully accomplished. Meanwhile, in a method using Nippou disk, the light is utilized effectively only by the amount of a ratio of a microscopic aperture in a uniform light. This condition requires a considerable

amount of time to be spent to detect the weak fluorescent light.

#### SUMMARY OF THE INVENTION

In order to solve the above-described  
5 problems, the present invention provides methods and units described hereinafter.

The inspection of a DNA chip to be inspected is executed in the following steps: Irradiating mutually different positions with a plurality of M  
10 excitation lights simultaneously with the use of an objective lens for a time  $\Delta t$  that is longer than a fluorescent light attenuation time, the excitation lights including a spot diameter  $d$  that is smaller than a dimension  $D$  of each cell, guiding obtained fluo-  
15 rescent lights to a fluorescent light detecting optical path, detecting the fluorescent lights on an image-forming plane that is in a conjugation relationship with irradiation spots onto the DNA chip, and executing the inspection of the DNA chip from positions and  
20 intensities of the fluorescent lights. At this time, the spot diameter is set to be substantially  $1/N$  of an integer  $N$  indicating the cell dimension. Moreover, relative positions between the irradiation spots and the DNA chip are caused to differ  $j$  times in sequence  
25 so as to inspect the entire inspection target positions  $LN^2 = Mj$ . Here,  $L$  denotes the cell number within the chip. Also,  $N$  is made equal to 2 or more and each cell

5 allows the fluorescent lights to be detected in a time less than  $LN^2/(6 \times 10^5)$  sec with reference to the entire inspection target sample-point number  $LN^2$  of the DNA chip.

There are provided M light-receiving aper-  
tures that have an effective diameter on the image-  
forming plane that is in the conjugation relationship  
with the plurality of M irradiation spots onto the DNA  
chip, the effective diameter being of substantially the  
same order as an effective diameter of the irradiation  
spot images. Moreover, an area other than the light-  
receiving apertures is light-shielded so as to detect  
the respective lights that have passed through the  
light-receiving apertures, thereby eliminating noise  
lights from the irradiation spots or from an area other  
than the irradiation spot plane so as to perform the  
inspection having a high signal-to-noise ratio. Also,  
the M light-receiving apertures that have the effective  
diameter are optical fiber light-receiving ends, the  
effective diameter being of substantially the same  
order as the effective diameter of the irradiation spot  
images. At the optical fiber light-receiving ends, the  
lights emitted from optical fiber light-emitting ends  
are detected, thereby performing the inspection having

an even higher signal-to-noise ratio.

The plurality of M irradiation spots onto the DNA chip are arranged on a straight line with a spacing of substantially  $kd$  with reference to the spot diameter  $d$  and an integer  $k$ . After the irradiation with this spot array during the time  $\Delta t$  has been performed, the spot array is displaced in an array direction by a distance of substantially  $d$ , and then the irradiation during the time  $\Delta t$  is performed. This operation is repeated  $k$  times in sequence, thereby performing the inspection concerning  $kM$  spot positions in the array direction. At the same time, the DNA chip and an inspecting apparatus are relatively displaced at least in a direction perpendicular to the array direction, thereby inspecting a desired 2-dimensional area of the DNA chip. Also, the above-described movement of the spot array is characterized by being performed using an acoustooptic deflector.

Also, it is desirable to set the integer  $k$  to be 2 or more. Furthermore, in view of the signal-to-noise ratio, it is more advantageous to set the integer  $k$  to be 5 or more.

The spot array is formed with the microlens array. The spot array can also be formed using a hologram. In synchronization with the above-described movement of the spot array in the array direction, a fluorescent light detection deflecting unit is provided within the fluorescent light detecting optical path so

that the fluorescent lights generated by the excitation lights come onto substantially the same location on the above-described light-receiving apertures. At this time, a deflecting unit using a piezoelectric device is employed as the fluorescent light detection deflecting unit. Also, the fluorescent light detection deflecting unit is configured with a wavelength selection beam splitter for permitting the excitation lights to pass through and reflecting the fluorescent lights. This makes it possible to perform the detection with a higher efficiency. Also, in order to enhance the separation from the excitation lights, a filter for permitting only the fluorescent lights to pass through and light-shielding the excitation lights is used within the fluorescent light detecting optical path isolated from excitation optical paths.

The above-described M multi excitation spot lights are configured so as to pass through substantially the same position A on a pupil of the objective lens. In addition, the excitation lights reflected regularly on the DNA chip are configured so as to reach a position B' on the pupil of the objective lens. In this way, a light-shielding unit for light-shielding the reflected excitation lights is provided at the position B' on the pupil of the objective lens, or is provided at an image position of B' that exists within the fluorescent light detecting optical path and exists on a plane conjugated with the pupil of the objective

lens. This allows the excitation lights becoming the noise components to be eliminated from a fluorescent light detection signal.

The above-described M multi excitation spot  
5 lights are configured so as to pass through substantially the same position A on the pupil of the objective lens. In addition, the excitation lights reflected regularly on the DNA chip are configured so as to pass through a position B that differs from the  
10 position A on the pupil of the objective lens. In this way, a light-shielding member for light-shielding the reflected excitation lights having a desired diameter with B as its center is located on the pupil of the objective lens, or is located at a position that is  
15 within the fluorescent light detecting optical path and is conjugated with the pupil of the objective lens. This configuration allows the excitation lights becoming the noise components to be eliminated from the fluorescent light detection signal.

20 Moreover, the reflected excitation lights to be light-shielded are employed as the regularly reflected excitation lights. The excitation lights scattered from the foreign substances within the DNA chip are extracted by being caused to pass through from  
25 outside the above-described light-shielding unit or the above-described light-shielding member. Then, the extracted scattered lights are branched off from the above-described fluorescent light detecting optical

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Employing, as an optical signal storage type

apparatus having an ultra-high sensitivity and including  $N_x \times N_y$  pixels, and assuming that  $n_x, n_y$  are integers,  $N_x \times N_y$  spots are simultaneously irradiated with the excitation lights of the spot diameter  $d$  with a pitch of  $n_x d$  in the  $x$  direction and with a pitch of  $n_y d$  in the  $y$  direction. Then,  $N_x \times N_y$  fluorescent light spot images obtained in this way are detected using the ultra-high sensitivity 2-dimensional image-pickup apparatus, and a relative position between the detecting apparatus and the DNA chip is displaced with a pitch of  $d$  in the  $x, y$  directions by the amount of  $n_x \times n_y$  steps. This method makes it possible to detect a desired area on the DNA chip.

Also, the above-described excitation lights, which include a plurality of different wavelengths, separate and detect a plurality of fluorescent materials-added different targets. Furthermore, the plurality of fluorescent materials-added different targets are simultaneously irradiated with the excitation lights including the plurality of different wavelengths, thereby separating and simultaneously detecting the different targets. This method makes it possible to detect various types of targets at high-speed.

On the inspection plane with which a desired fluorescent material-added target on the above-described DNA chip to be inspected has been hybridized, a second light is obliquely incoming into a proximity



to the above-described excitation spot lights and a position at which the second light is reflected on the inspection plane is detected, thereby detecting the focal point. A relative distance between the inspection plane and the above-described objective lens is controlled in accordance with this focal point information, thereby allowing the focusing to be achieved. Also, the obliquely launched second light is configured so as to pass through the objective lens. This permits the focal point detection and control to be executed with a simplified configuration.

The above-described obliquely launched second light is converted into an S-polarized light toward the detection plane. This enhances a reflectance on the fluorescent light detection plane, thereby making it possible to execute the correct focal point detection. In addition, as the wavelength of the obliquely launched second light, there is employed a wavelength that exerts no excitation to the above-described fluorescent material. This permits the precise detection to be executed without superimposing the noise on the fluorescent light detection signal.

It is an object of the present invention to provide a method and an apparatus therefor that obtain multi-spot lights having a substantially uniform intensity by solving a conventional problem that the light utilization efficiency in the conventional multi-spot light forming method is no good and there exist a

It is another object of the present invention to provide a method and an apparatus therefor that

In order to solve the conventional problem and obtain multi-spot lights positioned in proximity to each other, the present invention presents the following configuration:

As a concrete method of obtaining such doubled proximate beams, the plurality of N proximate laser beams are launched into the above-described polarization component including a multi-spot lights-doubling prism that has a polarization beam split plane

and total reflection planes. Here, the total reflection planes are parallel to the polarization beam split plane and are positioned at distances of  $L_1$  and  $L_2$  from the polarization beam split plane. This makes it possible to increase the number of the plurality of proximate laser beams up to the doubled number, i.e.,  $2N$ .

Also, the plurality of  $N$  proximate laser beams are launched into the above-described polarization component including an anisotropic optical medium. Then, in the anisotropic optical medium, each laser beam is separated into the two beams of the mutually orthogonal polarized components, thereby forming the plurality of  $2N$  laser beams. This makes it possible to form the plurality of  $N$  proximate laser beams into the plurality of  $2N$  laser beams which travel in substantially the same direction and the adjacent beams of which are in comparative proximity to each other.

In addition, the use of the above-described beam-doubling type polarization components in plural number  $M$  makes it possible to obtain  $2^M N$  laser beams.

The plurality of  $N$  proximate laser beams, which are launched into at least one of the above-described one or more of polarization components, are formed into converging laser beams, respectively. This allows the microscopic multi-spot lights to be obtained at a convergence position of the converging laser beams. At this time, the converging laser beams are

formed using lenses arranged in plural number, thereby forming the multi-spot lights.

Also, a mask, in which  $2^{M'}N$  or more of apertures having a diameter substantially equal to a convergence diameter of the multi-spot lights are arranged with reference to an integer  $M'$  satisfying  $1 \leq M' \leq M$ , is located at the convergence position of the above-described converging laser beams so that the multi-spot lights are focused onto the apertures. This makes it possible to obtain pure microscopic multi-spot lights without the noise lights or stray lights.

The above-described plurality of  $N$  laser beams can also be doubled even when the plurality of  $N$  laser beams have a  $4d$  or less of adjacent spacing pitch with reference to the beam diameter  $d$  of the laser beam emitted from the laser light-source or of the semiconductor laser beam formed into a parallel beam. Also, executing the doubling makes it possible to reduce the adjacent beam spacing further down to  $1/2^{M'}$ . Namely, without using the lens system, it is possible to form the multi-spot lights having the pitch smaller than the diameter  $d$  of the laser beam formed into the parallel light-flux.

As a method of forming the above-described plurality of  $N$  laser beams, there is provided one or more of 2nd beam-doubling prisms having a cross section of a triangle and a cross section of a parallelogram. Here, the triangle has an oblique side forming an angle

of substantially 45 degrees toward the light-emitting surface, and the parallelogram is in contact with this oblique plane and has a total reflection plane that is parallel to this oblique plane. This method, through  
5 the operation of the second beam-doubling prisms, makes it possible to form, for example,  $N/2$  laser beams into the plurality of  $N$  laser beams.

Moreover, there are used two or more types of the 2nd beam-doubling prisms having dimensions that are  
10 different from the dimension of the above-described 2nd beam-doubling prism. Then, these prisms are arranged from the largest to the smallest in sequence from the travelling directions of the beams in an  $n$ -stage cascaded manner, thereby  $2^n$  times multiplying the number  
15 of the beams. Namely, assuming that the dimension of the beam-doubling prisms at the final stage is equal to  $D$ , their number is set to be  $N/2$ , and the dimension and the number of the beam-doubling prisms at a stage preceding thereto are set to be  $2D$  and  $N/4$ , respec-  
20 tively, and the dimension and the number of the beam-doubling prisms at the first stage are set to be  $2^{n-1}D$  and  $N/2^n$ , respectively. This method allows the  $N$  laser beams to be obtained from an extremely small number of  $N/2^{n+1}$  laser beams.

25 The methods explained so far permits the multi-spot lights to be obtained from the laser beams emitted from the one laser light-source or the plurality of laser light-sources. What is more, using



lights incoming into the above-described polarization components are adjusted to the correct states, or a film thickness of a multi-layered film type polarization beam splitter is adjusted to the correct thickness. This allows the intensities of the doubled beams to be substantially equated with each other, thereby making it possible to restrain within  $\pm 10\%$  the variation in the respective spot energies among the multi-spot lights.

10           The above-explained  $2^M$  times multiplication of the number of the laser beams makes it possible to form the proximate multi microscopic spots. Thus, the multi-spot lights obtained in this way are used for the confocal point detection, the fluorescent light detection or the DNA detection or the like. This makes it possible to execute the conventionally impossible detection where the light utilization efficiency is high and the variation among the multi-spot lights is small, thereby eventually allowing the confocal point detection, the fluorescent light detection or the DNA detection or the like to be executed at a high-speed and with a high-accuracy.

25           The multi-spot lights formed using the above-described multi-spot light forming methods according to the present invention are projected onto an object to be observed. Then, the lights having been reflected at or having passed through the object to be inspected are caused to pass through multi apertures located at

positions at which the above-described projected spots are image-formed. Next, the lights having passed through the respective apertures are each detected individually. Executing the confocal point detection in this way makes it possible to execute the confocal point detection where the light utilization efficiency is high and the variation among the multi-spot lights is small, thereby implementing the high-speed and high-accuracy confocal point detection.

10           The multi-spot lights formed using the above-described multi-spot light forming methods according to the present invention are employed as excitation lights, then being projected onto the object to be observed that includes a fluorescent material. Then, 15 fluorescent lights generated by the excitation lights at the object to be inspected are separated from the excitation lights. Next, detectors are located at positions at which the fluorescent lights generated at the excitation light-projected spots are image-formed, 20 thereby detecting the fluorescent lights at the positions of the respective multi-spot lights. This allows the plurality of positions to be simultaneously irradiated with the excitation lights with a high-intensity. Also, since the variation among the respective spots is small, it becomes possible to execute a 25 high-speed and high-accuracy fluorescent light detection.

In the above-described fluorescent light



detection, the fluorescent lights are caused to pass through multi apertures located at the positions at which the fluorescent lights generated at the excitation light-projected spots are image-formed. In addition, there are located the detectors for individually detecting the fluorescent lights having passed through the respective apertures. This eliminates fluorescent lights other than the fluorescent lights generated by the excitation lights at the object to be observed, thereby making it possible to execute a fluorescent light detection having a high signal-to-noise ratio.

The multi-spot lights formed using the above-described multi-spot light forming methods according to the present invention are employed as the excitation lights, then being projected onto the object to be observed that includes a fluorescent material-added DNA. The target to be observed is implemented in the following way: A known probe DNA is attached to a predetermined position on a substrate such as a glass, and a target DNA is poured onto the glass substrate so as to hybridize the target DNA corresponding to the nucleotide sequence of the probe DNA, thus implementing the object to be observed. Here, the target DNA is formed by adding the fluorescent material to an end of the DNA purified and amplified from a living body sample, i.e., a target to be inspected. The excitation lights of the multi-spot lights are irradiated with the

In the above-described DNA inspection, the fluorescent lights are caused to pass through the multi apertures located at the positions at which the fluorescent lights generated at the excitation light-projected spots are image-formed, then individually detecting the fluorescent lights having passed through the respective apertures. This makes it possible to detect the fluorescent light intensities at the positions of the respective multi-spot lights so as to inspect the DNA, thereby being capable of executing the DNA inspection having an even higher signal-to-noise ratio.

In the fluorescent light detection and the DNA inspection, in many cases, a wavelength of the excitation lights and that of the fluorescent lights come comparatively close to each other. The use of the multi-spot lights in such a case enlarges the fields-

of-view in the detection and the inspection. This condition makes insufficient the characteristics of the optical systems for separating the fluorescent lights from the excitation lights, the optical systems being  
5 inserted halfway in the course of guiding, to the detecting systems, the fluorescent lights generated by the respective multi-spot excitation lights. Namely, the incident angles of the fluorescent lights incoming into the optical systems for separating the fluorescent  
10 lights from the excitation lights become different at the respective spots. Here, examples of the optical systems are the wavelength separating beam splitter and the interference filter that are installed halfway extending from the image-forming optical system to the  
15 detectors. As a result, the excitation lights to be shielded leak away depending on the location in the multi-spot lights, which makes the accurate detection impossible.

In order to solve the above-described  
20 problem, in the method of separating the fluorescent lights from the excitation lights, in addition to still using the wavelength separating beam splitter, the image-forming optical system is replaced by a telecentric image-forming optical system. In the  
25 telecentric image-forming optical system, primary light-rays of the fluorescent lights become parallel to each other, the fluorescent lights being generated from the respective spots extending from the image-forming

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optical system to the image-formed positions through the excitation by the respective multi-spot lights. Moreover, the interference filter and/or the wavelength separating beam splitter are inserted between the

5 telecentric image-forming optical system and the image-forming positions. This method causes the fluorescent light generated from whatever spot excitation light to be launched into the interference filter or the wavelength separating beam splitter at an incident angle

10 that is common to all the fluorescent lights. As a consequence, concerning the fluorescent light from whatever spot and the excitation lights becoming the noise components as well, it becomes possible to select the fluorescent lights and to eliminate the excitation

15 lights under the same condition, i.e., under an optimum condition. This situation allows the fluorescent light detection to be executed with an extremely small amount of the excitation light leakage, i.e., with an extremely small amount of the noise. As is seen from

20 the above-described explanation, the use of this fluorescent light detecting method further makes it possible to perform, precisely and with a high-accuracy, the DNA inspection where it is required to detect the fluorescent lights the intensities of which

25 are exceedingly weaker as compared with the intensities of the reflected excitation lights.

It is an object of the present invention to provide a distribution measuring apparatus for measur-

5 performance and the high-speed characteristic at the  
same time.

apparatus for scanning and reading a light-emission pattern from the fluorescent marker captured on a sample surface on a substrate. The fluorescent marker distribution measuring apparatus is characterized by including the following configuration components: A stage for mounting thereon the substrate of a target to be read, a unit for forming a plurality of irradiation spots on the sample surface on the substrate with a specified spacing that is larger than a diameter of the irradiation spots, a movement mechanism for moving the stage, a converging/detecting unit for converging and detecting light-emissions generated from the plurality of irradiation spots on the sample surface, a control unit for controlling the movement mechanism so that the substantially entire surface of the sample surface is irradiated in correspondence with the formation positions of the plurality of irradiation spots, and a processing unit for processing a signal from the converging/detecting unit so as to reconstruct images

on the sample surface. The intensities of the plurality of irradiation spots are set to be substantially almost equal to each other.

The unit for forming the plurality of  
5 irradiation spots can be configured as follows: The unit includes at least one laser light-source as excitation light-sources. Moreover, the unit splits a light emitted from an excitation light-source so as to image-form the irradiation spots in such a manner that  
10 the irradiation spots are arranged on the sample surface and a spacing of adjacent irradiation spots becomes smaller than 1mm. The unit for splitting the light emitted from the excitation light-source can be configured as the splitting prism, the optical anisotropic medium, the optical fiber, a polarization mirror  
15 or a combination of these devices.

The unit for forming the plurality of irradiation spots can also be configured as follows: The unit includes  $N$  ( $N \geq 1$ ) excitation light-sources and  
20  $M$ -stage 2-splitting units ( $M > 2$ ), and forms  $N \times 2^M$  irradiation spots. As the 2-splitting unit, there can be employed the splitting prism, the optical anisotropic medium, the optical fiber or the polarization mirror.

25 The unit for forming the plurality of irradiation spots can also be configured as follows: The unit includes a plurality of laser light-sources and a plurality of optical fibers. Furthermore, light-

emitting ends of the plurality of optical fibers are located on a straight line, thereby forming the plurality of irradiation spots.

It is preferable that the plurality of  
5 irradiation spots should be positioned on a straight line with a substantially fixed spacing.

The converging/detecting unit converges and, almost simultaneously, detects a plurality of fluorescent emitted lights generated from the plurality of  
10 irradiation spot-portions. The unit includes a wavelength selecting filter so as to separate fluorescent components from excitation components out of the emitted lights generated from the plurality of irradiation spot-portions, then detecting the fluorescent  
15 components. The converging/detecting unit can also be configured as follows: The unit has pinholes at which the emitted lights generated from the plurality of irradiation spot-portions are image-formed, then detecting the lights passing through the pinholes, the  
20 pinholes the number of which being the same as that of the irradiation spot-portions.

The control unit makes it possible to measure the entire plane of the sample surface by the following method: Employing, as a unit, step-like movements on a  
25 first microscopic width basis at  $n$  times and a step-like movement on a second width basis at one time subsequent thereto, the second width being greater in comparison with the first microscopic width, the stage

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is displaced in a specified one-axis direction. Then, this process is repeated, thereby scanning the sample surface. More concretely, the control unit is capable of controlling the stage movement so that the following operation is implemented: Employing, as the unit, (n-1)-times step-like style movements on the basis of a width of  $1/n$  (n: an integer) of the irradiation spot spacing and the subsequent one-time step-like style movement on the basis of (a width between both ends of the excitation spot group + the first width), the movement in the irradiation spots-arranged direction is executed, and this process is repeated thereby scanning the sample surface.

It is desirable that fetching of the signal from the converging/detecting unit should be executed while the stage is being displaced in a direction perpendicular to the plurality of irradiation spots-arranged direction.

The unit for forming the plurality of irradiation spots is required to form the plurality of irradiation spot-portions simultaneously. In order to image-form the lights onto the sample surface, there is employed a condenser lens the data of which is as follows: The numerical aperture is 0.7 or more, the effective field-of-view is 1.0 mm or more, and the difference between the sample surface and the lens tip is 0.7 mm or more.

The unit for forming the plurality of



The unit includes a plurality of optical anisotropic media the lengths of which in the optical axis direction differ from each other or a plurality of optical

The unit for forming the plurality of  
10 irradiation spots can also be configured as follows:

15 travelling direction is equal to about one-half of a  
length of an optical anisotropic medium located  
immediately preceding thereto.

20 The lights from the excitation light-sources are scanned and launched in sequence into one ends of a plurality of aligned optical fibers, and light-emitting ends of the optical fibers are located on a straight line, thereby forming the plurality of irradiation  
25 spots.

Also, it is preferable that there should be provided a function of executing an intensity correction for each detection signal of the respective

irradiation spots.

It is an object of the present invention to provide a technology for detecting weak fluorescent lights at a high-speed, with a high-resolution and with  
5 a high-sensitivity with reference to a target to be detected that requires 2-dimensional large number of lines of resolution. Also, It is another object thereof to provide, using this technology, a technology for inspecting a DNA with a high-sensitivity and at a  
10 high-speed.

In order to accomplish the above-described objects, the present invention employs the following methods and units:

The target object having a fluorescent  
15 characteristic is irradiated with multi-spot excitation lights including a large number of M microscopic spots. Fluorescent lights generated by the excitation lights from the respective multi spots are separated from the excitation lights. Then, fluorescent light images  
20 emitted from the target object are detected using a plurality of weak light detecting devices allowing the photon counting to be executed. This makes it possible to detect even an extremely weak fluorescent light. Photon signals obtained from the respective detecting  
25 devices are each photon-counted individually, then individually storing the photon-counted numbers  $N_{pm}$  detected by the respective detecting devices. In addition, positions of the multi-spot lights and a

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5 photon-counted number data over a desired range on the  
target object makes it possible to construct the fluo-  
rescent light picture from the collected data.

Also, sheet-shaped excitation lights are used instead of the above-described multi-spot lights.

10 Fluorescent lights, which have been generated from  
irradiation areas obtained by the irradiation with the  
sheet-shaped excitation lights and having a long and  
narrow configuration, are separated from the excitation  
lights. Then, fluorescent light images emitted from  
15 the target object are detected using the plurality of  
weak light detecting devices allowing the photon count-  
ing to be executed. Photon signals obtained from the  
respective detecting devices are each photon-counted  
individually, then individually storing the photon-  
20 counted numbers  $N_{pm}$  detected by the respective detect-  
ing devices. In addition, positions of the irradiation  
areas resulting from the sheet-shaped excitation lights  
and having the long and narrow configuration and the  
position of the target object are changed relatively,  
25 then storing in sequence the photon-counted numbers  
from the respective detectors. Storing and collecting  
in this way the photon-counted number data over a  
desired range on the target object makes it possible to

construct the fluorescent light picture from the collected data.

By executing the irradiation with the multi-spot lights or the sheet-shaped excitation lights and detecting the fluorescent lights from M pixels simultaneously, it becomes possible to spend a time of M to  $2M \mu\text{sec}$  per pixel in the case of trying to detect the  $6000 \times 6000$  pixels in 1 minute. Here, the case has been described previously in the conventional problems. For example, assuming  $M = 50$ , this time becomes equal to 50 to  $100 \mu\text{sec}$ . This condition makes it possible to solve the previously-described problem concerning the delay in the fluorescent light generation. Also, the following reason permits the dynamic range to be ensured and the high-sensitivity detection to be executed.

Namely, the previously-described per-pixel detecting time of the order of  $1 \mu\text{sec}$  shortens a detecting time that is long enough for a photo counting measuring time needed to execute the high-sensitivity detection, thereby making it difficult to ensure the dynamic range. This is attributed to the fact that, since the pulse-width of a photon pulse signal generated when one photon is detected is equal to tens of  $n$  seconds, only a signal of, at most, tens of pulses can be detected within the time of  $1 \mu\text{sec}$ . Also, even if, in such a short time, an attempt is made to detect the fluorescent light intensity in an analogue manner,

frequency characteristics of the detecting circuit make it difficult to execute the detection in a state where a sufficient dynamic range has been ensured.

Employing, as the above-described target  
5 object, a sample obtained from coupling a fluorescent molecule-added DNA fragment with a DNA corresponding thereto, the inspection is executed in the following way: In the case where the target to be inspected is, as an example, a DNA chip, a target that results from  
10 adding a desired fluorescent material to a DNA fragment formed from the target to be inspected, i.e., a DNA, is hybridized with the DNA chip at first. Then, the hybridized DNA chip to be inspected is irradiated with the multi-spot excitation lights including the large  
15 number of M microscopic spots. Fluorescent lights obtained by the irradiation from the multi spots are separated from the excitation lights. Next, separated fluorescent light images emitted from the DNA chip are detected using the plurality of weak light detecting  
20 devices allowing the photon counting to be executed. Moreover, photon signals obtained from the respective detecting devices are each photon-counted individually, then individually storing the photon-counted numbers  $N_{pm}$  detected by the respective detecting devices. In  
25 addition, positions of the multi-spot lights and a position of the DNA chip are changed relatively, then storing in sequence the photon-counted numbers from the respective detectors. In this way, the photon-counted

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A multi-spot aperture or a long and narrow aperture that permits only a multi-spot image or a sheet-shaped long and narrow area image to pass through is located at a focal point achieving position of fluorescent light images from the above-described target object surface, thereby performing the confocal point detection, the focal point achieving position being situated at a position at which the diameters of the multi-spot excitation lights or the sheet-shaped excitation lights with which the target object is irradiated become minimum-focused diameters. This makes it possible to obtain the confocal point image having a high signal-to-noise ratio. Namely, it becomes possible to detect, without being subjected to the influence from a fluorescent light noise and thus with a low-noise, the fluorescent lights at the multi-spot positions to be detected or the fluorescent lights at the sheet-shaped beam positions to be detected.

Here, the fluorescent light noise exists in the background of substances such as a fluorescent material existing in optical paths other than the above-described positions to be detected. If the sample  
5 toward which the fluorescent light detection is performed exists within a 2-dimensional plane, relative positions between the multi spots and the sample are changed within the plane, then performing the confocal point detection. This method allows the high signal-  
10 to-noise ratio fluorescent light detection or DNA inspection to be executed in a state of being scarcely subjected to the influence of a noise by a fluorescent material existing outside the plane.

In the above-described fluorescent image  
15 detection using the multi-spot lights, the relative positions between the multi spots and the sample are changed in at least one direction out of three directions in total, i.e., two directions within the target object surface and the remaining one direction  
20 perpendicular to the target object surface. In particular, in the case of the above-described confocal point detection, the relative position of the target is changed in the optical axis direction from the position at which the diameters of the multi spots or the sheet-  
25 shaped beams become the minimum-focused diameters, then determining the focal point achieving position from the obtained variation in the fluorescent light image intensity. Accordingly, from the fluorescent light

intensity at this position and toward a target having irregularity on its surface, it becomes possible to obtain the accurate fluorescent light intensity information, surface irregularity information, or information on the 3-dimensional positions emitting the fluorescent lights.

As having been pointed out in the problem in the case of the above-described conventional one-spot excitation light, the larger the number of the multi-spot lights becomes, the more advantageous it becomes to detect the fluorescent light image at a high-speed. Setting M to be 10 or larger increases, up to hundreds, the maximum photon-counted number detectable within this time, thereby becoming effective in implementing the high-sensitivity characteristic and the broad dynamic range. Since an even higher-speed characteristic is implemented in the DNA inspection and the like, providing the plurality number of spots exhibits an outstanding effect. Making M equal to 50 or more exhibits a more outstanding effect. Namely, setting M to be 50 or larger makes it possible to detect, for example, the 6000×6000 pixels in spending tens of  $\mu$  seconds per pixel, thereby allowing all the pixels to be detected within 1 minute.

The above-described multi spots are arranged on a 1 or 2-dimensional straight line in order to accomplish the following purposes: Detecting the multi spots using a plurality of detecting devices, above



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counting that is more than  $\alpha$  times as large as the value obtained by dividing the per-pixel detecting time by the photon pulse time-width. Accordingly, in the following way, the photon counting value is adjusted to

5 the value toward which the photon counting is effectively executable: Weakening the excitation lights, shortening the excitation light irradiation time to effectively lower the excitation light intensities, or lowering the gain of the detecting device so as to weak

10 the detected fluorescent lights.

The above-described multi-spot excitation lights or sheet-shaped excitation lights are converted into multi-color lights having 2 or more of wavelengths. This increases the information on the

15 fluorescent light detection, thereby enhancing the fluorescent light detecting function and the DNA detecting function.

Also, a time during which the above-described photon pulse signal is being positioned at a high level

20 is addition-measured during a time  $\beta T_d$  that is less than the per-pixel detecting time  $T_d$ . When setting the value of this time to be  $T_h'$ , it is judged whether or not a condition  $T_h' \geq \gamma \beta T_d$  is satisfied with reference to a predetermined  $\gamma$  that is smaller than 1.

25 If this inequality should be satisfied, there is employed a result obtained by integrating the output signal from the detecting device in an analogue-style integrating circuit. This makes it possible to perform

the detection toward stronger fluorescent lights as well. Namely, even in the case of detecting the strong fluorescent lights the photon counting of which is impossible, executing the analogue-style integration

5 allows the strong lights to be detected. What is more, making the analogue-style detecting time equal to  $T_d$  or less allows all the pixels to be detected without changing the per-pixel detecting time. As a consequence, it becomes possible to execute the detection

10 with a high-resolution and at a high-speed over a broad dynamic range ranging from the weak fluorescent lights necessitating the photon counting to the strong detection intensities necessitating the analogue integrating detection based on the above-described integrating

15 circuit. Also, as a detecting method where the above-described  $\alpha$  is equivalent to 1, there exists the following method: The photon counting and the analogue integrating detection are executed in parallel simultaneously so as to compare and judge both results

20 obtained, then employing one of the detected results.

Also, a laser light-source is used as the above-described excitation light-source. A multi-spot generating hologram or a sheet-shaped beam generating hologram is located within the laser resonator, and the

25 multi spots are generated using the strong laser light within the laser resonator. Usually, the optical energy within the laser resonator is more than 10 times as high as the emitting beam energy. In an ordinary

laser resonator, the reflectance of a mirror at the emitting window is set to be substantially 90%, and the remaining substantially 10% is extracted as the emitting light. The reflectance at the emitting window is  
5 made equal to 100% so as to confine the light within the resonator, and the hologram is located in an optical path within the resonator. This method allows substantially 10% of the hologram' diffraction efficiency to be utilized as the multi-spot lights or  
10 the sheet-shaped beams. As a result, it is possible to obtain the multi-spot lights or the sheet-shaped beams the intensities of which are almost 10 times higher as compared with those in the conventional case where the hologram is located in a laser-emitting optical path.  
15 Consequently, it becomes possible to execute the high-speed and high-sensitivity fluorescent light detection and DNA inspection.

The present invention permits a laser light emitted from a laser light-source to be converted into  
20 the multi-spot lights with a exceedingly high-efficiency and without being wasted. At the same time, the present invention makes it possible to extremely lessen the variation among the respective multi-spot lights. As a consequence, using the multi-spot lights,  
25 it becomes possible to execute the high-speed and high-accuracy confocal point detection. Also, using the multi-spot lights, it becomes possible to execute the high-speed and high-accuracy fluorescent light detec-

The present invention allows even the analogue integrating detection to be executed, thereby making it possible to execute the detection having a exceedingly broad dynamic range. Namely, it becomes possible to even detect the fluorescent light image of, for example, the 6000×6000 pixels in the dynamic range of  $2^{16}$  in the detecting time of 1 minute. As a result, it becomes possible to detect targets to be inspected, such as the DNA chip, in the detecting time of an order of 1 minute. This eventually makes it possible to detect, in a short time and precisely, a large quantity of samples to be inspected that are collected in a widely executed group checkup.

Also, the present invention is not only applied to the DNA inspection, but is widely used for a fluorescent light detecting microscopic method. The present invention tremendously shortens the times that have been required for the conventional observations and inspections. Namely, it becomes possible to detect, in a short time, even a weak light toward which only several photons can be detected. Also, it becomes possible to execute, in a short time and with a high-accuracy, the detection over a broad range ranging from the weak light to the strong light to be detected.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a diagram for illustrating an



receiving apertures on the fluorescent light detection plane in the embodiment in FIG. 1;

FIG. 11 is an embodiment-illustrating diagram in the present invention, where the multi spots are  
5 formed using a hologram;

FIG. 12 is a diagram for illustrating a method of creating the hologram in FIG. 11;

FIG. 13 is a diagram for illustrating another embodiment in the present invention;

10 FIG. 14 is a diagram for illustrating a 2-dimensional multi-spot generating microlens array used in the embodiment in FIG. 13;

FIG. 15 is a diagram for indicating a characteristic of the wavelength separating beam  
15 splitter in the embodiments in the present invention;

FIG. 16 is a diagram for indicating a characteristic of an interference filter in the embodiments in the present invention;

FIG. 17 is an embodiment-illustrating diagram  
20 in the present invention, where excitation lights are light-shielded using a space filter;

FIG. 18 is a diagram for illustrating the space filter in FIG. 17;

FIG. 19 is another embodiment-illustrating  
25 diagram in the present invention, which is a diagram for illustrating an excitation light light-shielding method utilizing the polarization;

FIG. 20 is an embodiment-illustrating diagram

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in the present invention, where the 1-dimensional spot array is detected using a 2-dimensional multi-channel photomultiplier tube;

FIG. 21 is a diagram for illustrating light-  
5 receiving and light-emitting ends of optical fibers and taking-in of spot images in the embodiment in FIG. 20;

FIG. 22 is an embodiment-illustrating diagram in the present invention, where the detection is executed using excitation lights having a plurality of  
10 different wavelengths;

FIG. 23 is an embodiment-illustrating diagram in the present invention, where a plurality of semiconductor lasers are used as the excitation lights;

FIG. 24 is an embodiment-illustrating diagram  
15 in the present invention, where multi-spot lights are obtained from light-emitting ends of optical fibers;

FIG. 25 is an embodiment-illustrating diagram in the present invention, where the multi-spot lights are obtained from one laser beam;

FIG. 26 is an embodiment-illustrating diagram  
20 in the present invention, where using excitation lights having a plurality of different wavelengths, the simultaneous detection is executed;

FIG. 27 is an embodiment-illustrating diagram  
25 in the present invention, where irradiation with the multi-spot lights is performed in an oblique direction and regularly reflected excitation lights are light-shielded on a pupil;



FIG. 28 is a diagram for illustrating a light-shielding portion on the pupil in FIG. 27;

FIG. 29 is an embodiment-illustrating diagram in the present invention, which indicates a method of  
5 detecting scattered excitation lights so as to determine a foreign substance position and correcting a fluorescent light detection result;

FIG. 30 is a diagram for illustrating the regularly reflected excitation lights and the scattered  
10 excitation lights between an objective lens and a DNA chip in FIG. 29;

FIGS. 31A, 31B, 31C are diagrams for indicating a method of the correction by a detection signal of the foreign substance-scattered excitation lights;

15 FIG. 32 is an embodiment-illustrating diagram in the present invention, which indicates focus detection and the control;

FIG. 33 is a diagram for illustrating a reflection response of the DNA chip in the focus  
20 detection in FIG. 32;

FIG. 34 is an embodiment-illustrating diagram in the present invention, where the focus detection is executed through the objective lens;

FIG. 35 is an embodiment-illustrating diagram  
25 in the present invention, which indicates a method of detecting the entire inspection target area of the DNA chip;

FIG. 36 is an enlarged explanatory diagram

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FIG. 45 is a perspective view for illustrating the configuration of an embodiment of the fluorescent light detecting and DNA inspecting apparatus

according to the present invention;

FIG. 46 is a front view for illustrating an embodiment of the multi-spot light forming unit according to the present invention;

5           FIG. 47 is a front view for illustrating an embodiment of a 2-dimensional multi-spot light forming unit according to the present invention;

FIG. 48 is a front view for illustrating an example of the arrangement of the 2-dimensional multi-spot lights according to the present invention;

FIG. 49 is a front view for illustrating an embodiment of a confocal point detecting apparatus according to the present invention;

FIG. 50 is a diagram for indicating the relationship between the multi-spot lights and stage scanning in the confocal point detection according to the present invention;

FIG. 51 is a schematic diagram for illustrating the entire configuration of one embodiment of a fluorescent marker distribution measuring apparatus according to the present invention;

FIG. 52 is a diagram for illustrating the configuration of an embodiment of a multi light-source distribution unit according to the present invention;

25           FIG. 53 is a diagram for illustrating the configuration of another embodiment of the multi light-source distribution unit according to the present invention;

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DNA chip for illustrating the case where there are employed the sheet-shaped excitation lights according to the present invention; and

FIG. 80 is a perspective view of a portion of an optical system for illustrating an embodiment where the multi spots according to the present invention are formed by a hologram within a laser resonator.

#### DESCRIPTION OF THE EMBODIMENTS

FIG. 1 is a diagram for illustrating an embodiment in the present invention. The reference numerals denote the following configuration components:

1 A multi-spot excitation lights-irradiating system for forming multi-spot excitation lights to irradiate a DNA chip 2 with the excitation lights so that a fluorescent light detection can be executed, 3 a fluorescent light detecting system for detecting fluorescent lights generated by the multi-spot excitation lights, 11 an excitation light-source including an excitation light light-source and an excitation light beam forming optical system. A He-Ne laser light beam is formed into a desired longitudinal-to-transverse ratio of the beam diameter by two cylindrical lenses having different focal lengths, then being launched into an AO deflector 12 through a mirror 1000. The AO deflector 12 includes a terminal of a frequency- $\omega$ high-frequency voltage applied to a quartz oscillator, and an input terminal of an amplitude signal the frequency

$\omega_v$  of which is lower than the frequency  $\omega$ .

The signal with the frequency  $\omega$ , which is transmitted from a control apparatus 4, has a frequency band in a range of  $\omega \pm \omega_0$ . Changing the frequency results in a change in the diffraction angle of the excitation light incoming into the AO deflector 12. Also, an inputting of the amplitude signal  $\omega_v$  from the control apparatus 4 brings about a change in the diffraction efficiency, thereby making it possible to control the diffraction light intensity. The diffraction light having passed through the AO deflector is separated from its 0th light (not illustrated) (the 0th light is light-shielded). Then, a lens system 13 including two lenses 131, 132 of focal lengths  $f_1$ ,  $f_2$  irradiates a microlens array 14 with the separated diffraction light in a desired beam diameter. Changing the frequency of the AO deflector 12 changes the angle of the excitation light incoming into the microlens array 14, but causes no change in the position thereof.

FIG. 4 is an enlarged detailed view of the microlens array 14. Microscopic microlenses composed of glass are arranged in a 1-dimensional configuration in a large number of 32 to 256. Let's explain the light incoming therein: For example, lights 1010 represented by solid-lines pass through the respective microlenses 141, 142, ... ..., then image-forming microscopic spots 111, 112, ... ..., 11M on a straight line  $\ell$  on a focal point plane  $\Sigma$ . As illustrated by



dotted-lines in FIG. 4, changing the frequency of the AO deflector 12 changes the angle of the excitation light incoming into the microlenses. As a result, the positions of the microscopic spots on the straight line 1 are changed to the positions as indicated by  $1j_1$ ,  $1j_2$ ,  $\dots$ ,  $1j_M$ .

As is illustrated in FIG. 1, through the use of a lens 15 and an objective lens 16, a fluorescent material added to targets hybridized with the DNA chip 2 is irradiated and excited with the microscopic-spot array  $11_1$ ,  $11_2$ ,  $\dots$ ,  $11_M$  formed on the focal point plane  $\Sigma$  of the microlens array 14. The focusing of the objective lens 16 is achieved so that the minimum beam diameter is formed on a glass surface  $\Sigma_1$  or  $\Sigma_2$  to which the targets have been attached (Concerning the details, refer to FIG. 33).

FIG. 2 illustrates the details of a surface structure of the DNA chip 2. Square-shaped minimum units  $20_1$ ,  $21_1$ ,  $20_2$ ,  $21_2$  and so on, which are indicated by longitudinal and transverse thin lines, represent detection pixels. In the drawing, a cell 20 is equivalent to  $5 \times 5$  pixels (indicated by a bold line). A fragment of the same DNA information is planted in one cell. Consequently, a target having the same DNA fragment structure is hybridized with this cell.

The reason why one cell is divided into a plurality of pixels is as follows: When a foreign substance such as a protein is mixed in one cell, in

some cases, an excitation light incoming into this protein generates a large-intensity fluorescent light. The dimension of such a foreign substance is usually equal to, at most, several  $\mu\text{m}$ . Accordingly, if the  
5 pixel dimension is equal to several  $\mu\text{m}$  and the cell dimension is equal to 10  $\mu\text{m}$  for example, and if a method as described later allows the foreign substance position to be detected and be separated within the cell of 10  $\mu\text{m}$ , it becomes possible to precisely  
10 determine the magnitude of the fluorescent light in accordance with the information on a portion other than the foreign substance portion.

FIG. 3 illustrates a side view of the DNA chip. The configuration given in this drawing is that  
15 the hybridized targets are mounted in a state of being naked on a glass substrate. This state is presented in some cases, and there are other cases where, as will be described later, the hybridized targets are sandwiched between glass substrates. In any case, the microscopic  
20 excitation light spots are converged onto the plane on which the targets exist. The light-converged diameter is substantially equal to the dimension of the pixel to be detected, i.e., the square-shaped minimum unit illustrated in FIG. 2.

25 At the initial frequency of the AO deflector 12, there has been obtained the diffraction light represented by solid-lines in FIG. 1. At this time, as illustrated in FIG. 3, the pixels 201, 202, 203, ... ..,

20M on the DNA chip are irradiated with the excitation light spot array as indicated by 101, 102, 103, ... ..., 10M. This irradiation, as will be explained later, allows fluorescent lights to be detected from the  
5 respective pixels. The irradiating time of the excitation light spots is equal to a time  $\Delta t$  (several to hundreds of  $\mu$ seconds) that is larger than the fluorescent light attenuation time. In the present embodiment, the irradiating time is equal to 60  $\mu$ s.

10 Moreover, after the lapse of 60  $\mu$ s, changing the frequency of the AO deflector 12 gives rise to the change in the diffraction angle of the excitation light. Then, as illustrated in FIG. 1 and FIG. 4, lights 10j,0 denoted by dotted-lines are launched into  
15 the microlens array 14. As a result, as illustrated in FIG. 3, the DNA chip is irradiated with the excitation light spot array as indicated by 111, 112, 113, ... ..., 11M. This irradiation allows fluorescent lights to be detected from pixels 211, 212, 213, ... ..., 21M. In this  
20 way, the irradiation with the M multi spots is executed in sequence with a shift on a pixel-pitch basis. When the shifts by the amount of j pixels have been finished, it turns out that the fluorescent lights by the amount of jM pixels have been detected all.

25 Next, referring to FIG. 1, the explanation will be given below concerning the embodiment of the fluorescent light detection. A beam splitter 30 located between the lens 15 and the objective lens 16

is a wavelength separating beam splitter. In the present embodiment, the wavelength of the He-Ne laser light from the excitation light-source is equal to 633 nm. The fluorescent material added to the targets on the DNA chip is Cy5, and the wavelength of the fluorescent lights to be detected lies in proximity to 670 nm.

The wavelength separating beam splitter 30 permits the 45°-incident excitation light at the wavelength of 633 nm to pass through by substantially 100%, and causes the 45°-incident fluorescent lights at the wavelength of 670 nm to be reflected by substantially 100%. The beam splitter, however, reflects the 633 nm excitation light as well by a exceedingly slight amount. The exceedingly slight amount of reflection becomes a problem since the fluorescent lights are extremely weak. Accordingly, in the embodiment in FIG. 1, an interference filter 34, which has its central wavelength characteristic at 670 nm and the half-value width of which is equal to about 15 nm, is inserted into the fluorescent light detecting system 3 so as to cause the interference filter to light-shield the excitation light leakage. Incidentally, the component 34 is not limited to the interference filter. Thus, it is allowable to use so-called a color filter for permitting a certain value or more of wavelength light to pass through and causing a certain value or less of wavelength light to be light-shielded. Also, the

combined use of the color filter and the interference filter is allowable. Hereinafter, the explanation will be given concerning the embodiments, using the interference filter alone for the simplicity of the explanation.

Next, the explanation will be given below concerning the following: Changes in the positions of detected fluorescent light images when the positions of the multi-spot excitation lights on the DNA chip are changed using the AO deflector, and corrections of the position changes needed in order to execute the detection with the fixed detector. The wavelength separating beam splitter 30 in FIG. 1 performs the corrections of the position changes as well. FIG. 5 illustrates the main portion in FIG. 1, and the same reference numeral as that in FIG. 1 denotes the same component. The wavelength separating beam splitter 30 is driven by a piezo device 301 having a high resonant frequency characteristic of 5 to 10 kHz, and is configured to perform a microscopic rotation with the y axis as its center.

FIGS. 6A to 6E and FIG. 7 are diagrams for explaining the role of this microscopic rotation, and the same reference numeral as that in FIG. 1 denotes the same component. By the deflection signal inputted into the AO deflector 12 from the control apparatus 4, the respective positions of the multi excitation microscopic spots 1011, 1021, ... ..., 10M1 on the DNA

chip 2 in FIG. 6C are changed to 1012, 1022, ... ..., 10M2 in sequence on the one-pixel basis. At this time, assuming that the wavelength separating beam splitter 30 performs no rotation, as illustrated in FIG. 6D, multi fluorescent light points 1211, 1221, ... ..., 12M1 on a fluorescent light detection plane  $\Sigma_{PH}$  that is located at a position conjugated with the DNA chip are changed to 1212, 1222, ... ..., 12M2 on the one-pixel basis as well.

10           The fluorescent light spot image 1212 on the fluorescent light detection plane, as illustrated in the drawings, is image-formed as a 1212 point onto an end of one optical fiber in an optical fiber bundle 322 by a fluorescent light detecting microlens array 321, then being launched into the one optical fiber. The one optical fiber is formed of a core 3221 through which a light passes and a portion 3222 for protecting the core. The diameter of the core through which the light passes is somewhat larger than that of the (multi) fluorescent light point 1211 on the fluorescent light detection plane  $\Sigma_{PH}$ . If, however, the wavelength separating beam splitter 30 performs no rotation, the 1211 point is displaced to 1212, 1213, 1214 ... ... (FIG. 6E) by the driving of the AO deflector 12, then being shifted from the optical fiber light-receiving end. This makes the detection impossible. Accordingly, as illustrated in FIG. 7, using the piezo device 301 having the high resonant frequency characteristic, the

wavelength separating beam splitter 30 is driven so that it performs the microscopic rotation.

Namely, in FIGS. 6A to 6E in the case of no microscopic rotation, the fluorescent light microscopic spot image 1211 is displaced to the different locations 1212, 1213, 1214. On the other hand, as illustrated within a dotted-line frame in FIG. 7, driving the beam splitter to perform the microscopic rotation permits the spot to come onto substantially the same position on the plane  $\Sigma_{PH}$ .

FIGS. 8A to 8F illustrate the relative time variations among the following respective signals:  $S_{A12}$ , an ON-OFF signal or an intensity modulation signal of the above-described AO deflector 12,  $S_{B12}$ , the deflection signal of the AO deflector 12,  $S_{30}$ , a driving signal of the piezo device 301 for deflection-driving the wavelength separating beam splitter 30,  $S_{33K}$ , a fluorescent light detection signal detected by one k-th photomultiplier tube 33 through a fiber 32,  $S'_{33K}$ , a per-pixel image storage (integration) signal of the fluorescent light detection signal, and  $S''_{33K}$ , a per-pixel final result of this image storage (a value obtained by a sampling-and-holding of  $S'_{33K}$  at a point-in-time before the image storing has been performed and the multi-spot excitation lights transfer to the excitation of the next pixel). In the embodiment of this graph, the multi-spot excitation lights are shifted in sequence on the one-pixel basis by the AO

deflector 12, and in this way, one spot has been shifted in sequence by the amount of ten pixels.

In order to enhance a signal-to-noise ratio in the detection of each pixel, the number of this shifting need to be equal to 2 or more. The larger the number is, the more desirable it is to implement the enhancement of the signal-to-noise ratio. However, conditions such as restrictions on the apparatus's configuration components naturally impose an upper limit on the number. Nevertheless, making the number equal to 5 or more outstandingly reduces the influences of the scattered lights or the fluorescent lights accompanied by the foreign substance irradiation with the adjacent multi-spot excitation lights halfway in the optical paths.

As illustrated in FIGS. 8A to 8F, the frequency (the frequency of an ultrasound applied to an ultrasound quartz oscillator, i.e., the deflection angle of the deflector) signal  $S_{B12}$  of the AO deflector 12 is changed in sequence in a step-like manner. During this change, the driving signal  $S_{30}$  of the piezo device 301 for deflection-driving the wavelength separating beam splitter 30 is changed linearly in response to this change. The AO deflector 12 diffracts a light by an extremely slight amount of change of a transparent medium's refractive index caused by the ultrasound propagation. Meanwhile, the piezo device 301 drives the beam splitter 30 as a whole. As a



result, there exists a difference in the frequency response characteristic between them. Consequently, the fast frequency signal  $S_{B12}$  of the AO deflector 12 is formed into the step-like configuration and the slow driving signal  $S_{30}$  of the piezo device is formed into the linear configuration.

FIG. 9 is a diagram for illustrating the positions of the fluorescent light spot images formed by these two signals on the fluorescent light detection plane  $\Sigma_{PH}$ . The change from the above to the below in this drawing represents the lapse of a time  $t$ . Two graphs on the left side indicate the changes of  $S_{B12}$  and  $S_{30}$ . Although the number of the steps is equal to 10 in FIG. 8, it is equal to 5 in this drawing. Solid-line circles 3211', 3221', 3231', ... that exist on the right side of the graph in FIG. 9 indicate the position shift of the fluorescent light detection multi-spot image in the case where, as was explained in FIGS. 6A to 6E, the position correction by the piezo device has not been performed yet. Incidentally, the right direction (transverse direction) in the drawing represents the arrangement direction  $x_a$  of the spot array, and solid-line circles 3212', 3222', 3232', ... indicate the position shift of the fluorescent light spot image adjacent to the above-described image. Moreover, dotted-line circles indicate the consequences obtained as the result of executing the position correction by the piezo device.

Namely, even when the AO deflector 12 had finished performing the step displacement of the excitation light and thus the excitation light has already stopped, the piezo device is still driving the deflection linearly. As a result, the corrected position of the fluorescent light spot image formed on the fluorescent light detection plane  $\Sigma_{PH}$  is displaced as is indicated by 3211<sub>-</sub>, 3211, 3211<sub>+</sub> by a slight amount though, while the excitation light continues to stop (The corrected position of the adjacent fluorescent light spot image is displaced as is indicated by 3212<sub>-</sub>, 3212, 3212<sub>+</sub> by a slight amount, though).

When the excitation light has been step-displaced to the next irradiation position on the DNA chip, and even if it continues to stop there, the corrected position of the fluorescent light spot image is displaced as is indicated by 3221<sub>-</sub>, 3221, 3221<sub>+</sub> by a slight amount, though (The corrected position of the adjacent fluorescent light spot image is displaced as is indicated by 3222<sub>-</sub>, 3222, 3222<sub>+</sub> by just a slight amount, though). In order to cover and detect this slight amount of displacement of the fluorescent light spot images, as illustrated in FIG. 10, there is provided an arrangement of ellipse-shaped apertures 3201, 3202, ... ..., 320M on a fluorescent light detection light-receiving plane (or, a plane conjugated with the light-receiving plane) 320, the longer radius of the ellipse being in the spot array direction  $x_A$ . In the

case of the above-described detection using the fiber, it is all right to set a condition that the fiber light-receiving end plane should include the ellipse.

FIG. 11 is a diagram for illustrating an embodiment in the present invention. The same reference numeral as that in FIG. 1 denotes the same component or the same function. In the present drawing, there is illustrated only a portion relating to the generation of the multi-spot excitation lights out of the entire apparatus in FIG. 1. The configuration components other than the portion are basically the same as those in FIG. 1. A reference numeral 14' denotes a multi-spot generating hologram.

FIG. 12 is a diagram for illustrating a method of creating the multi-spot generating hologram. As illustrated in FIG. 12, a laser light is irradiated with a mask 18 having pinhole array  $1j_11'$ ,  $1j_12'$ , ... ...,  $1j_1M'$  apertures having hole diameters corresponding to dimensions of the multi-spot excitation lights. Then, the transmittance lights having passed through the mask are converged onto a hologram storage medium by a Fourier transformation lens 15. Next, the converged position is irradiated with a reference light 1000 from a somewhat oblique direction at an incident angle  $\phi = \phi_0$  in a superimposed manner, thereby forming the Fourier transformation hologram at the above-described converged position.

In order to enhance the light utilization

efficiency, there is employed a phase-modulation type storage medium. Also, random phases that are independent of the mutual positions of the respective apertures are added to the apertures. This addition is performed  
5 in order to avoid the following situation: The intensity of a 0th light at the central position (a position where the space frequency is equal to 0) on the Fourier transformation surface is increased up to an order-of-magnitude difference, and thus values such  
10 as the signal-to-noise ratio and the diffraction efficiency of the formed hologram become worse.

The hologram created in this way is used in the multi-spot excitation lights-generating system in the DNA inspecting apparatus in FIG. 11.

15 In FIG. 11, a laser light is launched into the AO deflector 12 and then passes through the lens system 13. Next, the hologram 14' that has been created by the above-described method is irradiated with the laser light at an incident angle  $\phi_0$ . An  
20 incident angle  $\phi$  of the laser light launched into the hologram is set so that, at the center (the center of the deflection angle) of the driving frequency of the AO deflector 12, the incident angle  $\phi$  becomes equal to the incident angle  $\phi_0$  of the reference light 1000  
25 toward the hologram surface at the time of creating the hologram by the method in FIG. 12. The irradiation of the hologram with the laser light in accordance with this setting, as illustrated in FIG. 11, reproduces, at

$1j_1, 1j_2, \dots, 1j_M$ , a spot array that is the same as the pinhole array at the time of creating the hologram in FIG. 12.

Next, changing the frequency of the AO deflector brings about a slight amount of change in the incident angle into the hologram, thereby bringing about a slight amount of change in the angles of the multi-spot reproduced lights as well. As a result, it turns out that a position equivalent to the adjacent pixel is illuminated and excited. Accordingly, a sequential driving of the AO deflector makes it possible to execute the multi-spot excitation illumination of the DNA chip in the step-like manner, i.e., in such a manner that the position to be irradiated is changed in sequence on the one-pixel pitch basis.

FIG. 13 is a diagram for illustrating another embodiment of the DNA inspecting apparatus in the present invention. The same reference numeral as that in FIG. 1 denotes the same component. In the present embodiment, unlike the embodiment in FIG. 1, the detection in a 2-dimensional manner is executed as well simultaneously. Namely, the excitation illumination light is caused to pass through an illuminating lens 13' capable of simultaneously illuminating a 2-dimensionally-broad range. Moreover, the excitation illumination light is reflected by a wavelength selection beam splitter 30', then illuminating a 2-dimensional microlens array 14'. The 2-dimensional

microlens array 14' has the same functions as those in the case where the 1-dimensional microlens array has been already explained using FIGS. 1 to 4, and what differs therefrom is the 2-dimensional array arrangement alone. Consequently, as illustrated in FIG. 14, 2-dimensional microscopic spots 1410' are formed at focal point positions of microlenses 141'.

In the case of the present embodiment, pinhole apertures are bored with the positions of 1410' as their center. In this way, the DNA chip is irradiated with only the lights that have passed through the pinhole apertures. A ratio between a diameter  $d$  of the microscopic multi spots and an arrangement pitch  $p$  of the spots is equal to an integer that is larger than 2 and that, preferably, is larger than 5. As illustrated in FIG. 13, a high-resolution lens 16' causes the DNA chip to be simultaneously irradiated and excited with the 2-dimensional multi-spot lights having passed through the pinhole apertures. The pixels undergoing the excitation irradiation, i.e.,  $2x11y11$ ,  $2x12y11$ ,  $2x11y21$ ,  $2x21y11$ , ... have an equal pitch spacing with 4 pixels skipped.

Fluorescent lights generated from the above-described pixels that have simultaneously undergone the excitation irradiation pass through the above-described high-resolution lens 16', then passing through the respective pinholes 1410' on the lower surface of the microlens array. The fluorescent lights having passed

through the pinholes pass through the respective micro-  
lenses, thereby being enlarged up to a size of the  
upper surfaces (convex surfaces) of the microlenses.  
The fluorescent light intensities on the upper surfaces  
5 of the microlenses are image-formed onto a high-  
sensitivity image storage type 2-dimensional sensor 32'  
through the wavelength selection beam splitter 30' and  
an image-forming lens 31'. Although the interference  
filter is not illustrated in the present drawing, the  
10 interference filter, or the color filter that permits a  
wavelength longer than the fluorescent lights to pass  
through is provided between the wavelength separating  
beam splitter 30' and the 2-dimensional sensor 32'.

In the 1-dimensional and 2-dimensional  
15 microlens arrays illustrated in FIG. 4 and FIG. 14,  
respectively, a light incoming into an intermediate  
area between the adjacent microlenses in the respective  
microlenses is in a danger of becoming a scattered  
light and then a noise light. Thus, by performing a  
20 masking (not illustrated) by which the intermediate  
area is covered with a light-shielding portion composed  
of a material such as chromium oxide, it becomes  
possible to eliminate such a noise.

FIG. 15 and FIG. 16 illustrate a spectro-  
25 scopic reflection characteristic and a spectroscopic  
transmittance characteristic respectively of the wave-  
length separating beam splitters 30, 30' and the  
interference filter 34, which have been used in the

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As the fluorescent material added to the DNA fragment to be hybridized, several types of fluorescent substances are employed. For example, when employing often employed Cy5 (Cyanine5), an absorption peak wavelength of the fluorescent material is equal to 649 nm and a peak wavelength of the fluorescent lights is equal to 670 nm. Also, on an even shorter wavelength side, when employing Cy3 (Cyanine3), the absorption peak wavelength of the fluorescent material is equal to 550 nm and the peak wavelength of the fluorescent lights is equal to 570 nm. Since the spectroscopic absorption characteristic of the absorbing material has a bandwidth, it is not always required to cause the wavelength of the excitation laser light to coincide with the absorption peak wavelength. Accordingly, there is used a laser light the wavelength of which is close to the absorption peak wavelength.

In the case of Cy5, there is used a red He-Ne



laser light at the wavelength of 633 nm or a semiconductor laser light at the wavelength of 635 nm. In the case of Cy3, there is used a green He-Ne laser light at the wavelength of 544 nm or the like. In the interference filter and the wavelength separating beam splitter for extracting only the fluorescent lights, as the central wavelength, a wavelength is selected which is close to the fluorescent light peak wavelength and at which it is easy to separate the excitation lights.

10 In the above-described fluorescent light detection, it becomes of the utmost importance to completely light-shield the excitation lights especially when the fluorescent lights are weak.

FIG. 17 is an embodiment-illustrating diagram in the present invention, where it is intended to execute the above-described light-shielding of the excitation light more completely. Namely, the light-shielding based on the present embodiment is executed in the following cases: The use of the interference filter or the wavelength separating beam splitter alone is insufficient for the light-shielding, or it is desirable to increase the band half-value width of the interference filter in order to increase the fluorescent light detection intensities. In FIG. 17, the same reference numeral as that in FIG. 1 have the same component or the same function.

A multi-spot array  $111, \dots, 11M$  formed on a plane  $\Sigma_f$  by the microlens array or the hologram is

image-formed onto the DNA chip as excitation lights through the operation of the lens 15 and the objective lens 16. At this time, the respective multi-spot lights pass through a center of an incident pupil  $EP_0$  of the objective lens with a spread the radius of which is substantially  $RNA_{\sigma}$ . Setting a numerical aperture and the focal length of the objective lens to be NA and  $f$ , respectively, a spot diameter  $D_s$  of the excitation lights on the DNA chip is given by the following equation:

$$D_s = 2k_1 f \lambda / RNA_{\sigma}' \quad (\text{where } k_1 = 0.6)$$

Representing, by  $p$ , a pixel resolution (the pixel pitch) for the DNA chip detection, this value is substantially equal to  $D_s$ . Assuming that  $p$  is equal to  $2 \mu\text{m}$  and the excitation light wavelength  $\lambda$  is equal to 633 nm, a value  $RNA_{\sigma}'/f$  becomes equal to 0.19. The value  $RNA_{\sigma}'/f$  is a NA of an illumination for illuminating the spot of  $2 \mu\text{m}$  (i.e., a minimum NA of the objective lens needed in order to image-form the  $2 \mu\text{m}$  spot). Representing this value by  $NA'$ , the  $NA'$  is given as follows:

$$NA' = \sin(\tan^{-1} (RNA_{\sigma}' / f)) \doteq RNA_{\sigma}' / f = 0.19$$

In order to detect the weak fluorescent lights, the NA of the objective lens 16 falls in the range of 0.7 to



conjugated therewith) is configured with lens systems 311, 312 as illustrated in the drawing. This configuration is designed so as to image-form the objective lens pupil at a position existing between 311 and 312.

- 5 A space filter 35 is located at this position conjugated with the pupil. The space filter 35 has a configuration as illustrated in FIG. 18.

The light-forming magnification of the objective lens pupil' surface toward the space filter 35 by the lens system 311 is assumed to be 1 time. The space filter has a circle-shaped light-shielding portion 351 of a radius  $RNA_{\sigma}$  in the central portion of an aperture, the aperture having a radius  $RNA$  that is equal to a radius of the objective lens pupil from the optical axis center of a light-shielding portion 353. The multi-spot excitation lights, which had been regularly reflected by the DNA chip, turns out to have the beam radius of  $RNA_{\sigma}$  on the space filter. Consequently, if  $RNA_{\sigma} > RNA_{\sigma}'$  holds, the regularly reflected excitation lights are light-shielded by the light-shielding portion 351.

Meanwhile, the fluorescent lights excited and generated by the multi-spot excitation lights are launched into the objective lens with almost no directivity, then incoming into the space filter. The light-shielding portion 351 light-shields the fluorescent lights as well, but a ratio of the light-shielded fluorescent lights toward the space filter

incoming fluorescent lights becomes equal to  $(NA'/NA)^2$ . Substituting the above-described value, this value is found to be 7 (when  $NA = 0.7$ ) to 4 (when  $NA = 0.9$ ) %, which is a loss of a negligible order.

5                   In this way, it is possible to reduce the unnecessary excitation lights tremendously without lowering the light amount of the fluorescent lights to be detected. The fluorescent light detection lights having passed through the space filter pass through the  
10 lens 34 and the interference filter 34, then image-forming the spot images on the DNA chip onto the detection plane, i.e., the optical fiber light-emitting ends, so as to be subjected to the fluorescent light detection.

15                   FIG. 19 is an embodiment-illustrating diagram in the present invention, which indicates a method of preventing the excitation lights from being guided into a fluorescent light detecting optical path. The same reference numeral as that in FIG. 1 denotes the same  
20 component. In the present embodiment, a polarization beam splitter 30" is employed as a beam splitter for branching off the fluorescent light detecting optical path from an excitation light optical path. Namely, a split surface of the polarization beam splitter is  
25 irradiated with the excitation lights that are P-polarized toward the split surface. Then, since the excitation lights having been reflected on the DNA chip surface and having returned back still remain P-

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polarized, the excitation lights pass through the polarization beam splitter and never enter the fluorescent light detecting optical path. On the other hand, since the polarization state of the generated  
5 fluorescent lights differs from that of the excitation lights, the S-polarized fluorescent lights are reflected by the polarization beam splitter 30", then being guided into the fluorescent light detecting optical path. In this way, this method makes it  
10 possible to prevent the excitation lights from being guided into the fluorescent light detecting optical path.

FIG. 20 is an embodiment-illustrating diagram in the present invention, where the fluorescent light  
15 detection is executed using the optical fibers and the multi-channel photomultiplier tube. Namely, FIG. 20 illustrates the concrete content of the detection of the fluorescent light multi-spot images in the embodiments illustrated in FIGS. 1, 5, 7, 17, and 19. As  
20 illustrated in FIG. 21, the light-receiving end of the fiber system 32 for the fluorescent light multi-spot images the multi-spot number of which is M or more is the multi-lens array 321 including 1-dimensionally arranged M lenses.

25 As having been explained in FIG. 7, using the respective microlenses, the fluorescent light multi-spot images are launched into the cores through which a light passes in the fibers. If, as illustrated in FIG.

20 the multi-channel photomultiplier tube 33 includes  
2-dimensionally arranged light-receiving apertures, the  
light-emitting ends of the fibers are also configured  
to be 2-dimensionally arranged. In the case of the  
5 embodiment in FIG. 20, the fluorescent lights emitted  
from the respective light-emitting ends are image-  
formed by the image-forming lens 324 in such a manner  
that the fiber light-emitting ends 323 correspond to  
the respective light-receiving apertures 331 of the  
10 photomultiplier tube 33.

In the case of the embodiment in FIG. 21, a  
2-dimensional lens array 3241 is provided at the light-  
emitting ends of the fibers in the corresponding  
manner. The fluorescent lights emitted from the  
15 respective fibers pass through the respective lenses,  
thereby being directly converged onto 2-dimensional  
light-receiving apertures (photoelectric plane) 3311 of  
the multi-channel photomultiplier tube. In the embodi-  
ments illustrated in FIGS. 20, 21, the 2-dimensional  
20 multi-channel photomultiplier tube is employed. When  
using the 1-dimensional multi-channel photomultiplier  
tube as well, configuring the light-emitting ends to be  
1-dimensionally arranged also allows the detection to  
be executed by the same method.

25 FIG. 22 is an embodiment-illustrating diagram  
in the present invention. In the present embodiment,  
as the excitation lights, there are used excitation  
lights having a plurality of different wavelengths.

Light-source systems 11A, 11B, and 11 C form lights emitted from excitation light light-sources with different wavelengths  $\lambda_A$ ,  $\lambda_B$ , and  $\lambda_C$ , then causing the lights to be launched into fiber bundles. In the

5 light-source systems, multi-lens arrays are arranged at the light-emitting ends, and pinhole arrays are provided at the positions at which the lights after being emitted are substantially converged, and the excitation lights having been emitted from the pinhole

10 arrays are configured so that the excitation lights pass through wavelength separating beam splitters 30A, 30B, and 30 C. As illustrated in FIG. 22, when the system 11A is selected as the excitation light light-source system for the fluorescent light detection, the

15 excitation light with  $\lambda_A$  passes through the lens 15, and is reflected by a deflection mirror 300, then passing through the objective lens 16 so as to perform multi-spot excitation irradiation toward the DNA chip

2. Moreover, fluorescent lights generated from the

20 respective multi spots pass through the objective lens 16, the deflection mirror 300, and the lens 15 (31). Then, the fluorescent lights are reflected by the wavelength separating beam splitter 30A, eventually passing through an interference filter 34A so as to be

25 detected by the method explained in FIGS. 20, 21 using the multi-channel photomultiplier tube.

When the fluorescent lights to be detected are of different types, the entire light-source system



50 (i.e., 11A, B, C; 120A, B, C; 130A, B, C; 30A, B, C;  
34A, B, C) is displaced in solid-line arrow directions  
in accordance with a control apparatus 4' by a driving  
mechanism not illustrated. Then, either of the  
5 different wavelengths  $\lambda_b$  and  $\lambda_c$  is selected so as to  
execute the fluorescent light detection using this  
selected wavelength. When the plurality of types of  
fluorescent lights are to be used for one DNA chip, the  
light-source systems are displaced in sequence, thereby  
10 executing the detection using the different excitation  
lights one after another.

FIG. 26 is another embodiment-illustrating  
diagram, where the above-described plurality of types  
of fluorescent lights are used. Unlike the case in  
15 FIG. 22, irradiations with the plurality of types of  
excitation lights are performed simultaneously, thereby  
trying to shorten the detecting time. In FIG. 26,  
excitation lights, which are emitted from light-source  
systems 11A', 11B', and 11 C' with a plurality of  
20 different wavelengths  $\lambda_a'$ ,  $\lambda_b'$ , and  $\lambda_c'$  corresponding  
to, for example, 3 colors of red, green, and blue, are  
synthesized effectively into one optical path by wave-  
length selection synthesizing mirrors 51, 52. Namely,  
the wavelength selection synthesizing mirror 51 permits  
25 blue to pass through and causes green to be reflected.  
Also, the wavelength selection synthesizing mirror 52  
permits red to pass through and causes green and blue  
to be reflected.

The synthesized 3 colors pass through a beam splitter 30' and, after passing through the lens 15, the deflection mirror 300, and the objective lens 16, perform 3-color simultaneous spot excitation illuminations with the 3 colors onto the DNA chip. The respective fluorescent materials are excited at each wavelength and emit lights with the respective fluorescent colors. The fluorescent lights emitted have wavelengths that are slightly longer than those of the excitation lights. This condition makes it possible to separate, using wavelength separating beam splitters 53, 54, the wavelengths of the 3-wavelength fluorescent lights excited by the 3-wavelength excitation lights. Namely, the wavelength separating beam splitter 53 causes the blue fluorescent light to be reflected and permits the green and the red to pass through. Also, the wavelength separating beam splitter 54 causes the green to be reflected and permits the red to pass through.

In optical paths resulting from the separation into the respective 3 colors in this way, there are located interference filters 34C, 34B and the filter 34A that each permit the respective fluorescent lights alone to pass through with a high-purity. Then, the microscopic spot images of the respective fluorescent lights are detected simultaneously by the above-described method using multi-channel photomultiplier tubes 33C', 33B', and 33A' through fibers.

In the embodiments in FIGS. 22, 26, the deflection mirror 300 performs the array direction scanning of the excitation multi-spot lights. As the deflection mirror, there is employed a mirror of the piezo driving system or of the galvanometer mirror type. In this case, unlike the embodiment explained in FIG. 1, the multi spots are subjected not to the step displacement but to a continuous (linear) scanning. The multi spots on the DNA chip are scanned, but one and the same (identical) deflection mirror is used in the detecting optical path as well. As a result, unmoved multi spots are image-formed at the ends of the fluorescent light detecting fibers. On account of this, it turns out that pixel addresses are determined based on the deflection angle of the deflection mirror.

Based on the relationship between the pixel addresses and the deflection angle, a plurality of fluorescent light detection signals obtained from the multi-channel photomultiplier tubes in a parallel manner are organized and stored by the control apparatuses 4' (FIG. 22) or 4" (FIG. 26). Such data processing, of course, requires that the measuring and detecting conditions be inputted in advance. These pieces of input information are inputted from a terminal 41' or from a high-order computer 40 then, as necessary, data on the measured and inspected result is transferred to the computer.

In the embodiment in FIG. 26, the explanation

has been given concerning the case where the irradiations with the plurality of excitation lights having the different wavelengths are performed simultaneously. By the way, in the case where there exist an overlap  
5 between the wavelength bands of the plurality of excitation lights and those of the fluorescent lights at which the respective excitation lights aim, a shutter situated near the light-sources and not illustrated is used toward the overlapped excitation  
10 light or the fluorescent light detection is executed with the time shifted by an ON-OFF of the light-sources themselves.

FIG. 23 is an embodiment-illustrating diagram in the present invention, where a plurality of semiconductor lasers 111A2, 111A2, ... having substantially  
15 the same wavelength are employed as the excitation light light-source. The semiconductor laser is of a small volume, producing a comparatively high-output, and is inexpensive. As a consequence, as illustrated  
20 in the drawing, configuring a multi-spot light-source using a large number of semiconductor lasers makes it possible to irradiate the DNA chip with intense multi-spot excitation lights, thereby allowing the high-speed detection to be implemented. The lights emitted from  
25 the respective semiconductor lasers are taken into light-receiving ends of fibers 120A1, 120A2, ... by lenses 112A1, 112A2, ...

The laser lights emitted from the light-

emitting ends, as illustrated in FIG. 24, are converged onto a pinhole arrangement 111, 112, ... through a microlens array. Then, the transmittance lights having passed through the pinhole arrangement are used as the  
5 multi-spot excitation lights.

If the semiconductor lasers are not usable as the excitation lights, there are used high-output solid-state lasers excited with the semiconductor lasers, and high-output gaseous lasers. In such laser  
10 light-sources, by using an emitted beam in such a manner as to be split by a method illustrated in FIG. 25, it becomes possible to form multi-spot excitation lights having substantially the same intensity and substantially the same beam configuration.

15 Namely, the beam emitted from a laser light-source 111A' is split using multi-splitting beam splitters 1110A', 1120A', ... . It is assumed that the number of the splitting is equal to k and reflectances of beam-split lights from the 1st stage of the splitting through the 2nd stage, the 3rd stage, ... to the  
20 k-th stage are equal to  $r_1, r_2, \dots r_j, \dots r_k$ . Considering that  $r_k$  is equal to 1 and since the respective beam-split lights are equal in their intensities, with reference to an arbitrary j ( $1 \leq j \leq k$ ),

25 
$$r_j = 1 / (k - j + 1)$$

should be satisfied in order to form the above-

described multi-spot excitation lights. Namely, at 1110A', since  $k$  is equal to 4, i.e., 4-splitting,  $r_1$ ,  $r_2$ ,  $r_3$ , and  $r_4$  become equal to  $1/4$ ,  $1/3$ ,  $1/2$ , and 1, respectively. Similarly, since 3-splitting is

5 performed at 1120A',  $r_1$ ,  $r_2$ , and  $r_3$  become equal to  $1/3$ ,  $1/2$ , and 1, respectively. In this way, the laser lights having the same intensity and the same beam configuration are launched into a lens array 112A1', then being launched into cores of the fibers.

10 In the embodiment in FIG. 23, the plurality of semiconductor lasers are employed as the excitation light light-source for the generation of the multi-beam spots. In the case where the available laser light power is inadequate, however, it becomes required to  
15 form the multi-spot excitation lights with the use of a plurality of gaseous lasers other than the semiconductor lasers or a plurality of lasers based on the 2nd harmonics. In such a case, the systems illustrated in FIG. 25 are used in plural number and the fiber light-  
20 emitting ends picked up from the plurality of systems are arranged on a 1-dimensional line or a 2-dimensional plane. This makes it possible to obtain the multi-spot excitation lights with a high-excitation intensity.

In the above-described case where the  
25 plurality of excitation lights are used, depending on the excitation wavelength, there arises a necessity of using various types of different lasers, such as the semiconductor laser, the gaseous laser, and the solid-

state laser using the 2nd harmonics excited with the semiconductor. In such a case, it is all right to form the multi-spot excitation lights in accordance with the above-described method illustrated in FIG. 23 or FIG.

5 25.

FIGS. 27, 28 are embodiment-illustrating diagrams in the present invention. In the drawings, by eliminating the influences of the fluorescent lights and the scattered lights generated by the excitation multi-spot lights from a foreign substance mixed in the DNA chip, or the influence of a scattered light from a foreign substance in the fluorescent light detecting optical system, it is made possible to implement the high-accuracy fluorescent light detection. A reference numeral 11' denotes an excitation light-source, which causes a light to pass through a pinhole aperture array 14' through a fiber so as to create the excitation multi-spot lights. The multi-spot lights are image-formed onto the DNA chip through the operation of the lens 15 and the objective lens 16. A space filter 161 is located at the position of an incident pupil of the objective lens.

The excitation multi-spot lights are set so that the multi-spot lights pass through a position 163 (refer to FIG. 28) shifted off from a center of the incident pupil. Namely, as illustrated in FIGS. 29, 30, the respective multi-spot lights pass through a portion shifted off from an optical axis of the





eliminating the noise caused by the excitation lights, which has been explained in the embodiment in FIG. 17.

In the embodiment in FIG. 27, in addition to this effect, the influences of the foreign substance mixed on or in the DNA chip are eliminated in the following way: Namely, if there exists the foreign substance such as various kinds of proteins mixed at the time of forming the DNA sample, the irradiation of the foreign substance with the excitation lights causes the excitation lights to be scattered, because the dimension of such a foreign substance is small, i.e., several  $\mu\text{m}$ . Also, in the case of the above-described foreign substance of the organic substance type, intense fluorescent lights are emitted from the foreign substance, becoming the fluorescent lights that are more intense than the fluorescent material added to the DNA to be detected.

As illustrated in FIG. 30, the scattered excitation lights pass through a dotted-line surrounded area  $L + S$  determined by the NA ( $= \sin\theta_{L+S}$ ) of the objective lens. Then, after passing through the objective lens, the scattered excitation lights pass through a portion other than the light-shielding member 162 of the space filter, leaking into the fluorescent light detecting system. Only this leakage of the scattered excitation lights is extracted using a wavelength separating beam splitter 61, then executing the image detection. The wavelength separating beam

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splitter permits the fluorescent lights to pass through and causes the excitation lights to be reflected. Although, in FIG. 29, the wavelength separating beam splitter 61 is inserted in optical paths behind the  
5 wavelength separating beam splitter 30", it may be inserted in optical paths before the beam splitter 30". In this way, in much the same manner that the fluorescent light image is photographed by a detector 33' such as the multi-channel photomultiplier tube, an  
10 excitation light scattered image S100 ((refer to FIG. 29) of the excitation lights scattered by the foreign substance is detected as an image by a detector 62.

FIGS. 31A, 31B indicate, at a signal level on the per-pixel basis, a fluorescent light image signal  $D_L$   
15 and an excitation light image signal  $D_S$  that have been detected in this way. Namely, numerals 1, 2, ... ..., 25 denote detected pixel addresses and numerals (1), (2), (3), (4), (5) denote addresses of cells to which the same DNA sequence is hybridized. Namely, for example,  
20 the pixels at the addresses 1, 2, 3, 4, 5 belong to the cell (1). Although, actually, the pixels in the cells are 2-dimensionally located and there exist, for example,  $5 \times 5$  pixels, the pixels are 1-dimensionally located in order to make the drawings-accompanying  
25 explanation easy to understand. If the detection is exerted upon a pixel where the foreign substance exists, the scattered excitation light from the pixel exceeds a threshold value  $D_{ST}$ .

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In FIG. 31B, at the pixel addresses 9, 10, 11, 12, and 16, 17, 18, 19, 20, the excitation light image signal  $D_s$  exceeds the threshold value  $D_{st}$ . Consequently, the information on the fluorescent light image signal  $D_L$  existing at the corresponding addresses is strongly influenced by the foreign substance. Accordingly, an average value of the fluorescent lights in each cell is determined by discarding the information at these addresses. Namely, concerning the cell (2), the average value is determined not by using the information at the addresses 9, 10 but by using only the information at the remaining addresses 6, 7, 8. Similarly, concerning the cell (3), the average value is determined by using only the information at the addresses 13, 14, 15. In the cell (4), the scattering from the foreign substance occurs in all the pixels, and thus this cell is regarded as being invalid. The average intensity in each cell is determined in this way as is illustrated below in the graph in FIG. 31C. This allows the influences of the foreign substance to be reduced tremendously, thereby making it possible to execute the precise fluorescent light detection.

Incidentally, in the optical systems of the embodiments explained using FIGS. 27 to 30 where the influences of the foreign substance are eliminated, there are provided the optical systems where the excitation lights illuminate the DNA chip after passing through the objective lens. However, the configuration

is also allowable where the irradiation with the excitation lights is performed from between the objective lens and the DNA chip in an oblique direction without passing through the objective lens. In this case, since the regularly reflected excitation lights are not launched into the objective lens, a space filter (which is equivalent to 161) becomes unnecessary.

FIG. 32 is an embodiment-illustrating diagram in the present invention. The reference numerals denote the following components: 1 the multi-spot excitation lights-irradiating system the detailed embodiments of which have been explained already, 3 the fluorescent light detecting system for detecting the fluorescent lights generated by the multi-spot excitation lights, the details of which have been also explained already. The fluorescent material attached to the DNA hybridized onto the DNA chip 2 is irradiated with the microscopic multi-spot excitation lights of several  $\mu\text{m}$  through the use of the objective lens 16. In order to accomplish this, it is required to always maintain the distance between the objective lens and the fluorescent light plane at a fixed spacing within the focal depth so that the spot size is kept at a constant value. For the purpose of this, there is used a focal point detecting system 7 that is structurally integrated with the objective lens 16 for implementing the rigidity. The focal point detecting system 7

includes an oblique beam spot irradiating system 71 and a spot position detecting system 72. The oblique beam spot irradiating system 71 irradiates the fluorescent light plane on the DNA chip with the microscopic spots from an oblique direction.

The irradiation with the spots is performed, and the spacing between the objective lens 16 and the fluorescent light plane is detected. Then, the objective lens 16 is controlled so as to be driven in the z direction in accordance with the detection signal by an objective lens up-and-down driving mechanism 73 so that, even if a stage 80 for mounting the DNA chip 2 thereon is driven in x, y directions by a driving motor 83(84), the above-described fixed spacing is maintained. A numeral 82 denotes a length-measuring device of x, y stage.

FIG. 33 illustrates an example of a cross sectional structure of the DNA chip. In this embodiment-illustrating diagram, the fluorescent light plane is a plane  $\Sigma_2$ . Namely, there exists the fluorescent light plane  $\Sigma_2$  on a flat substrate 23 such as a glass. Between this plane and a plane  $\Sigma_1$  on a glass substrate 21, there exists a clearance 22 into which a liquid containing the fluorescent material-added DNA to be inspected is poured for implementing the hybridization. At the stage of the DNA inspection, the clearance is filled with the liquid. However, depending on the cases, the clearance is left empty. Also, in some



outside the light-receiving aperture.

Namely, in this embodiment, it becomes possible to capture only the regularly reflected light reflected from the plane  $\Sigma_2$  and thus to detect the spot position on the position sensor, i.e., the height position of the plane  $\Sigma_2$ . Then, based on this detected information and in accordance with the control apparatus 4, the objective lens and the focal point detecting system 7 integrated with the objective lens are displaced slightly in an up-and-down direction by the driving mechanism 73. This allows the fluorescent light detection to be executed always in the focus-achieving state.

If the incident angle of the oblique incidence focus detecting light in FIG. 33 is close to Brewster angle, causing the detecting light to be launched in the P-polarized state gives rise to an extremely small reflection on the plane surface, thereby making it difficult to execute the detection. Accordingly, there is employed the S-polarized detecting light. The employment of the S-polarization enhance the reflectance on the plane surface more in comparison with that of the P-polarization no matter what value the incident angle is. This is advantageous enough.

FIG. 34 is an embodiment-illustrating diagram in the present invention, where the focal point detection is executed through the objective lens 16. A

laser light having been guided by a fiber 74 from a near-infrared semiconductor laser light-source not illustrated, after being emitted from the fiber, passes through a beam splitter 76, a lens 77, and a wavelength separating beam splitter 300". Then, the light passes through the objective lens 16 so as to be converged, and the fluorescent light plane on the DNA chip 2 is irradiated with the converged light from oblique directions. The incident angle  $\theta$  of this irradiating light is equivalent to the numerical aperture NA of the objective lens in its magnitude (an incident angle that is somewhat smaller than  $\sin\theta = NA \div 0.8$ ). The regularly reflected light passes through the objective lens again, eventually forming, onto a position sensor 73', an image of the light-converged point on the DNA chip 2. Then, the position sensor 73' detects the position of this image. From this detected position, the height of the plane in the DNA chip can be determined. The objective lens is displaced in the up-and-down direction so that the image is image-formed at a reference point on the position sensor 73'. This, in the same way as the embodiment in FIG. 33, makes it possible to detect the focus position of the fluorescent light plane in the chip. Just like the present embodiment, by employing the light the wavelength of which is longer than that of the detected fluorescent light as the light to be used for the focal point detection, it becomes possible to execute the focal



point detection precisely without exciting the fluorescent material, i.e., without generating the detection noise.

Incidentally, the wavelength separating beam splitter 300" in FIG. 34 permits the near-infrared light to pass through and reflects the excitation light and the fluorescent light used for the fluorescent light detection. The irradiation with a 1-dimensional excitation light spot array formed by a 1-dimensional excitation light irradiating optical system 1 is performed toward the fluorescent light plane in the DNA chip through the lens 15, the wavelength separating beam splitter 300", and the objective lens 16. A fluorescent light detecting optical system 3 detects the fluorescent light generated. The displacement of the excitation light spot array irradiating positions in the array direction is executed by causing the wavelength separating beam splitter 300" to perform the microscopic rotation.

Hereinafter, referring to FIGS. 35 to 40, the explanation will be given concerning a method of executing, all over the entire cells in the DNA chip, the fluorescent light detection toward the DNA chip that has been explained so far using the above-described respective embodiments.

FIG. 35 illustrates the entire structure of the DNA chip. A numeral 204 denotes the whole case for housing the DNA chip. The DNA chip is a glass

substrate on an inner side of a window 200 formed on this case, and the chip is fixed to the case 204. The fluorescent material-added DNA fragment is hybridized with an area 202 on the inner side of the window. In an area spreading outside the area 202 and inside the window 200, there are described alignment marks 201 for the positioning.

The relative positions between the cell 20 explained in FIG. 2 and the alignment marks 201 are designed and formed with an accuracy of several-tenth  $\mu\text{m}$ , the cell 20 including  $N \times N$  (in FIG. 5,  $5 \times 5$ ) pixels (indicated by the bold line) in which the fragment of the same DNA information is planted (i.e., the probing is performed).

The DNA chip is mounted on the detecting apparatus the embodiments of which have been explained already. Then, in an alignment detecting optical system (not illustrated) implemented in the detecting apparatus, using a device such as a mark position detecting 2-dimensional CCD, the positions of at least 2 of the alignment marks 201 are detected as positions on the CCD. Also, the position of the DNA chip at the time of executing this mark detection is detected using, for example as is illustrated in FIG. 32, the length-measuring devices 81, 82 for detecting the position in the x and y directions. The length-measuring devices are provided on a chuck on which the DNA chip is mounted.

The spacing between the optical axis of the above-described CCD detecting optical system and the detecting optical axis in the previously-described various types of fluorescent light detecting optical systems is fixed. This condition allows the pixels to be detected at the right positions from the above-described spacing, the above-described alignment mark detected positions by the CCD, and the detected position by the length-measuring devices, the pixels corresponding to more minute division of the inside of each cell on the DNA chip. At this time, if the detection of 2 or more of the alignment mark positions indicates that the DNA chip has been rotated, this rotation is corrected using a rotation mechanism not illustrated. Incidentally, the right position detection after this rotation correction is performed as necessary. Even if this rotation correction is not performed, if the amount of the rotation is small, it is also allowable to detect this rotation amount by the above-described method and to correct the x, y coordinates in accordance with the rotation amount detected. Also, it is possible to correct and detect the above-described multi-spot lights by a microscopic rotation of the optical system.

As having been explained so far, the detection of the alignment marks and the length-measurement of the DNA chip make it possible to detect the pixels in each cell on the DNA chip with the precise position-

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ing accuracy. As a consequence, in accordance with the following method, it becomes possible to execute the fluorescent light detection toward all the pixels in the chip in sequence, at a high speed, and without missing. A reference numeral 2A in FIG. 35 indicates an area detected by one scanning of the chuck. Here, the one scanning of the chuck is executed as follows: An operation of performing the irradiation with the M multi-spot array excitation lights, of performing the sequential scanning by the amount of N pixels in the spot array direction, and of returning the spot array back to an original operational position is repeated, and at the same time, the chuck is scanned in the y direction. Namely, as illustrated in FIG. 36, first, the pixels 2A101, 2A102, ... ..., 2A10M on the DNA chip are illuminated and excited simultaneously. Next, the multi spots are displaced by the amount of one pixel-pitch  $\Delta P$  by the above-described method, and this is continued in sequence.

When, in this way, the displacement by the amount of  $N(k)$  pixels is over, it turns out that the detection has been performed by the amount of  $MN(k)$  pixels in total, i.e., over a width of  $NM\Delta P$ , in a 1-dimensional array-like configuration. When the scanning by the amount of  $N(k)$  pixels has been finished, the multi-spot array is returned back to the original position. During this, the DNA chip has been displaced by the amount of one pixel in the y direction, which

corresponds to a time-period from a time  $t_0$  to  $t_1$  of  $y_{st}$  in FIG. 39. Consequently, during the time-period from the time  $t_0$  to  $t_1$ , the repetition of the above-described operation by the number of the y direction pixels  
5 allows the fluorescent light detection to be finished toward all the pixels in the area 2A.

In the case where the above-described operation is performed by the DNA detecting apparatus of the embodiment in FIG. 1, the driving signal of the  
10 multi-spot excitation lights by the AO deflector, or the displaced position of the spots is changed as is indicated by  $S_{px}$  in FIG. 38. When seen in more detail, the driving signal or the displaced position is step-displaced just like  $S_{B12}$  in FIG. 8. Also, the deflection  
15 signal of the wavelength selection beam splitter is changed similarly to  $S_{px}$  in FIG. 38. The repetition of such changes from the time  $t_0$  to  $t_1$  permits all the area of the area 2A to be detected.

When the detection has been performed to the  
20 end at the bottom of the area 2A (the time  $t_1$ ), as is indicated by  $x_{st}$  in FIG. 40, the DNA chip chuck is step-displaced over the width of  $NM\Delta P$  in the x direction. After the step displacement, as is indicated by  $y_{st}$  after the time  $t_1$  in FIG. 39, the chuck is scanned in an  
25 opposite orientation in the y direction. Simultaneously with this, just like  $S_{px}$  after the time  $t_1$  in FIG. 38, the deflection signal of the AO deflector 12 and that of the wavelength selection deflecting beam

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splitter 30 are scanned in an orientation opposite to the orientation at the time of detecting the area 2A (the time  $t_0$  to the time  $t_1$ ). This, as illustrated in FIG. 37, makes it possible to continuously detect an  
5 area 2B subsequently to the detection of the area 2A. By repeating the above-explained operation alternately in the - orientation and the + orientation concerning the y direction of the DNA chip, it becomes possible to detect all the areas 2A to 2J on the DNA chip at a  
10 high-speed.

Next, the explanation will be given below concerning a time needed to detect all the pixels on the DNA chip. Representing the cell number in the chip by L, each cell is divided into the  $N \times N$  pixels so as to  
15 avoid the influences of the foreign substance and the like. This makes the total detection pixel number in the chip equal to  $LN^2$ . Setting the simultaneous irradiation spot number of the multi-spot excitation lights and a time needed for the detection with the  
20 multi spots to be M and  $\Delta t$ , respectively, and ignoring a time for the spot displacement and a time for the chuck movement in the x direction since these times can be assumed to be shorter as compared with the fluorescent light detecting time, the total fluorescent  
25 light detecting time T is given by the following equation:

$$\begin{aligned} T &= LN^2 \Delta t / M \\ &= LN^2 / (M/\Delta t) \end{aligned}$$

With reference to the number  $k$  of the scannings with the multi spots, a response time  $T_p$  of the wavelength separating beam splitter 30 needs to be  $k\Delta t$  or larger. Also, the light-source power needs to be  $\beta M/\Delta t$  or larger. Also, it is desirable that the spacing between the excitation multi-spot lights be equal to 5 or more in view of the signal-to-noise ratio.

At a longer fluorescent light wavelength of 670 nm, the quantum efficiency of the photomultiplier tube used for the fluorescent light detection becomes considerably small, i.e., 5 to 10%. Also, there exists a limit to the output of the laser light used for the excitation. Taking such conditions into consideration and in order to execute the detection as fast as possible, letting, for example,  $k (= N) = 10$ ,  $M = 50$ ,  $\Delta t = 50\mu s$ , the cell number  $L = 1000 \times 1000$ , and the in-cell division pixel number  $N = 5$ ,  $T$  becomes equal to 25s. Namely, this result satisfies the condition of the fluorescent light detecting time that is smaller than  $LN^2 / (6 \times 10^5) \text{ sec}$ . Additionally, substituting the above-described values of  $L$  and  $N$  into this condition, the result is found to be 42 sec. This value satisfies the condition of executing the detection within 1 minute, including attachment/detachment of a sample chip for the use other than the fluorescent light

detection, inputting of the inspection conditions, and outputting of the result.

Allowing the detection to be executed in 1 minute exhibits an outstanding effect in the case where  
5 a lot of specimens to be detected are inspected. For example, 1 minute becomes sufficient to execute an inspection that has necessitated 5 minutes conventionally. Accordingly, even if a preprocessing spends somewhat a longer time, when the number of the  
10 specimens becomes around 100, it becomes usually possible to execute the preprocessing in parallel to the inspections of the large number of specimens. This makes it possible to shorten the detecting time by 5 or 6 hours.

15 The reasons why the present invention permits such a high-speed and high-accuracy detection to be implemented are as follows: The multi spots are used as the excitation lights simultaneously. Moreover, in the fluorescent light detection by each multi-spot  
20 excitation light, the detection is executed in a state of enlarging the spot spacing enough so that the influences of the other excitation spot lights can be suppressed up to the smallest possible degree.

FIGS. 41A, 41B, 41C, and 41D are diagrams for  
25 illustrating an embodiment of the method and units for obtaining the multi-spot lights according to the present invention.

Using a method described later, substantially



parallel laser beams emitted from one light-source or a plurality of light-sources are formed into a plurality of (in the drawing, an even number)  $N/2$  laser beams that are parallel to each other with a pitch of several 5 mm and that travel in the same direction, then being formed into  $N$  laser beams ( $N$  is an integer) by  $N/2$  beam-doubling prisms 411, 412 and so on. As illustrated in an enlarged perspective view in FIG. 48B, the beam-doubling prism has a cross section of a triangle 10 and a cross section of a parallelogram. Here, the triangle has an oblique side forming an angle of substantially 45 degrees toward the light-emitting surface, and the parallelogram is in contact with this oblique plane and has a total reflection plane that is 15 parallel to this oblique plane.

A light launched into the one side of the parallelogram is reflected on a beam split plane of the triangle' oblique plane by the amount of substantially 50%, and the remaining substantially 50% passes through 20 the beam split plane. The light having passed through the beam split plane passes through the prism of the triangle without being subjected to any change. Meanwhile, the reflected light undergoes a total reflection on the other plane of the parallelogram, then passing 25 through the prism of the parallelogram. In this way, the light having passed through the triangle prism and the light having passed through the parallelogram prism are parallel to each other and have substantially the

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same intensity. Also, the spacing between both of the lights becomes equal to  $P$ . As a result, the beam spacing between the adjacent multi beams has become equal to  $P$ , which is one-half of the beam spacing  $2P$  of the laser beams 81e.

In this way, by a beam-doubling prism array 4e where such beam-doubling prisms are arranged by the number of  $N/2$  with the pitch of  $2P$ , the laser beams 81e are formed into the plurality of  $N$  laser beams 8e which travel in substantially the same direction with the pitch of  $P$  and the adjacent beams of which are proximate to each other. The plurality of  $N$  laser beams 8e, by being launched into a polarization component 11e described later, are formed into a plurality of  $2N$  laser beams which travel in substantially the same direction and the adjacent beams of which are proximate to each other.

As illustrated in FIG. 42, the polarization component 11e includes the 1st multi-spot lights-doubling prism that has a polarization beam split plane 110e and transparent prisms 111e, 112e. Here, the transparent prisms each have total reflection planes 1101, 1102 that are parallel to the polarization beam split plane 110e and are positioned at distances of  $L1$  and  $L2$  from the polarization beam split plane 110e. On a light-receiving plane of the polarization component 11e toward the  $N$  beams, there are located strip-shaped convex lenses such as 21e, 22e, which result from

cutting a spherical flat convex lens into strip-shaped configurations, by the number of N in such a manner as to correspond to the positions of the respective N parallel beams. Here, the enlarged diagram and the cross sectional enlarged diagram of the strip-shaped convex lenses are illustrated in FIGS 49A to 49D and FIG. 42, respectively. The laser beams having passed through an array 20e of these strip-shaped convex lenses, as illustrated in FIG. 42, become converged lights each, then being launched into the polarization beam split plane in parallel to each other.

The respective converged beams are circularly polarized lights or linearly polarized lights polarized at 45 degrees toward the polarization beam split incidence plane 110e. Consequently, as illustrated in FIG. 42, an S-polarized component of an incident light 82e is reflected on this plane, and a P-polarized component thereof passes through this plane. As illustrated in FIG. 42, a reflected converged light 822 passes through the prism 111e, then being launched into the plane 1101 parallel to the polarization beam split plane 110e at an incident angle of 45 degrees. A converged beam 822' that has undergone a total reflection on the plane 1101 is S-polarized, and thus is reflected on the beam split plane 110e again. A reflected converged beam 822" passes through the prisms 111e, then going through.

Meanwhile, with respect to the P-polarized

component, a converged beam 821 having passed through the beam split plane 110e passes through the prism 112e, then being launched into the plane 1102 parallel to the beam split plane 110e at an incident angle of 45  
5 degrees. The reflected light becomes a totally reflected converged beam 821', then being launched into the beam split plane 110e again. The incident light is P-polarized, and thus passes through the beam split plane by substantially 100%. This transmittance light  
10 821" passes through the prism 111e, then going through.

The total reflection planes 1101, 1102 of the prisms 111e, 112e are positioned at the distances of L1 and L2 from the beam split plane. The values of L1 and L2 satisfy the following relation:  $\sqrt{2} (L2 - L1)$  is  
15 equal to  $P/2$  that is one-half of the pitch P of the N parallel beams 8. Accordingly, when the lights having been separated by the beam split plane go through the polarization component, the two lights are shifted to each other by the amount of  $P/2$ . As a result, the N  
20 converged beams with the pitch of P that have been launched into the polarization component 11e pass through the polarization component 11e in a state of being formed into 2N converged beams with the pitch of  $P/2$ .

25 On a light-emitting plane of the prism 111e in the polarization component 11e, as illustrated in FIG. 43, there is located a pinhole array mask 2 where pinhole apertures 211e having a diameter d are arranged

by the number of  $2N$  with a pitch of  $P/2$ . The converged lights pass through the pinhole apertures. Since the portion other than the  $2N$  pinhole apertures light-shields lights, the pinhole array mask 2 light-shields  
5 a stray light differing from the converged lights and becoming a noise light. As a result, the lights having passed through the pinhole array mask generate spot array lights only from the pinhole portions.

The lights having passed through the pinhole  
10 array 2e are linearly P-polarized lights and linearly S-polarized lights alternately every adjacent spots. Directly behind the pinhole array 2e, there is located a quarter wave plate 3e. The quarter wave plate is set in such a manner that the optical axis forms 45 degrees  
15 toward the P-polarized and S-polarized directions. Consequently, the P-polarized and the S-polarized lights having passed through the quarter wave plate 3e become right-handed and left-handed circularly polarized lights, respectively.

20 The  $2N$  parallel circularly polarized lights having passed through the quarter wave plate 3e are launched into a polarization component 12e. The polarization component 12e is a polarization beam splitter the structure of which is substantially the  
25 same as that of the above-described polarization component 11e. Namely, a beam split plane 120e permits a P-polarized light to pass through and causes an S-polarized light to be reflected. Also, the values of

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L1' and L2' satisfy the following relation:  $\sqrt{2} (L2' - L1') = \pm P/4$ . Here, L1' denotes a distance between the beam split plane and a total reflection plane 1201 of a prism 121e through which the S-polarized light passes  
5 after having been reflected by the beam split plane. L2' denotes a distance between the beam split plane and a total reflection plane 1202 of a prism 122e through which the P-polarized light passes after having been reflected by the beam split plane. By providing the  
10 above-explained configuration, the 2N parallel beams with the pitch of P/2 that have been launched into the prism 12e are formed into mutually parallel 4N diverged beams with a pitch of P/4, then being emitted from the prism 12e.  
15 As described above, the lights emitted from the 2N spots existing on the pinhole array mask are formed into the above-described 4N multi-spot lights by the polarization component 12e. At this time, the polarization component 12e makes the 4N multi-spot  
20 lights look as if the multi-spot lights were emitted from the surface of the mask 2e. Namely, when seen from the side on which the multi-spot lights have already passed through the polarization component 12e, the 4N multi-spot lights are formed on the surface of  
25 the mask 2e, then being emitted therefrom in the state of having become the diverged lights. This phenomenon is caused by a mirror image effect by the reflection planes 120e, 1201, and 1202.

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As one example, assuming that the number of N is equal to 16 and the pitch P is equal to 1.6 mm, in the above-described embodiment, the number of the multi-spot lights and the pitch become equal to 64 and 0.4 mm, respectively. By launching the multi-spot lights into an objective lens of an infinite focal point microscope through an image-forming lens, assuming that the magnification of the optical system is 20 times, it becomes possible to irradiate a sample, i.e., a target to be inspected, with 2  $\mu$ m-diameter spots with a 20  $\mu$ m pitch by the number of 64 simultaneously. This will be explained in detail later.

In the above-described embodiment in FIG. 41A, the N/2 multi beams are launched into the 2nd beam-doubling prism array 4e. When N is equal to 8, if the laser light-source is not the He-Ne laser type producing a large output but a large intensity is required as the irradiation light, it is sufficient to prepare, for example, 8 He-Ne lasers and to launch the lasers into the 2nd beam-doubling prism array 4e with a 1.6 mm pitch. Also, it is sufficient that 8 semiconductor lasers are used and are formed into mutually parallel beams, thereby configuring 8 beams as is the case with the above-described He-Ne lasers so as to launch the lasers into the 2nd beam-doubling prism array 4e. In this case, since the pitch P is set to be 1.6 mm, the spacing 2P between adjacent beams of the 8 laser beams becomes equal to 3 mm, which is greater

than the beam diameter of the He-Ne laser light, i.e., 1 mm. Accordingly, it is possible to launch the lasers in parallel to each other from the 8 laser light-sources.

5                   In this way, even in the state where, by the 2nd beam-doubling prism array, the pitch is made equal to the spacing  $P$  that is less than 4 times as great as a diameter  $d$  of the beams emitted from the laser light-source, i.e.,  $4d$  or less, causing the beams to be  
10   launched into the multi-spot light forming unit using the polarization component allows the spacing to be made equal to  $1/2$  thereof. Also, using the polarization component a plurality of  $n$  times allows the spacing to be made equal to  $1/2^n$  thereof. In this way,  
15   it becomes possible to execute the conventionally difficult following task: Converting the plurality of  $N$  laser beams, which are launched with the pitch that is less than 4 times as great as the beam diameter  $d$ , into the  $2^M N$  multi laser beams with the pitch of  $P/2^M$ ,  
20   thereby allowing the multi-spot lights to be formed with a high-efficiency.

FIG. 44 is a diagram for illustrating another embodiment for forming the multi-spot lights according to the present invention. In the present embodiment,  
25   the  $N$  multi beams to be launched into the polarization component are formed using one laser light-source. For example, in the case where, like YAGSHG laser, the output of the light-source is large, the one laser

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light-source is effective enough. In such a case, as illustrated in FIG. 44, in addition to the 2nd beam-doubling prism denoted by 4e in FIG. 41A, the 2nd beam-doubling prisms the dimensions of which are 2 times, 4 times, and 8 times the dimension of the above-described 2nd beam-doubling prism 4e are arranged in a cascaded manner at  $n = 4$  stages in total.

Namely, one beam 80e emitted from one light-source is launched into the 8-times-dimensioned beam-doubling prism 441, thereby obtaining 2 beams 801 and 802 with a spacing of  $8P$ . The 2 beams are launched into the 4-times-dimensioned beam-doubling prisms 431 and 432, thereby obtaining 4 beams 8011, 8012, 8021, and 8022 with a pitch of  $4P$ . The 4 beams are launched into the 2-times-dimensioned beam-doubling prisms 421, 422, 423, and 424, thereby obtaining 8 beams with a pitch of  $2P$ . The 8 beams are launched into the previously-mentioned 8 beam-doubling prisms 411, 412, 413, 414, 415, 416, 417, and 418 illustrated in FIG. 41B, thereby obtaining 16 beams with a pitch of  $P$ . This allows the 16 beams with the 1.6 mm pitch to be obtained from the one beam emitted from the one laser light-source.

The method explained in the above-described embodiment makes it possible to form the multi-spot lights having a total energy that is 70% or more of the energy of the laser beam emitted from the laser light-source, which was impossible in the prior art.

Moreover, taking the following measures makes it possible to form the multi-spot lights having a total energy that is 90% or more of the energy of the laser beam emitted from the laser light-source: Equip-  
5 ping the optical components with an antireflection coat, providing the optical components with a multi-layer coat for lowering the loss at the time of the beam separation, performing positioning of the incident lights toward the pinhole array, or the like.

10 Furthermore, the method explained in the above-described embodiment makes it possible to restrain the variation in the respective spot energies among the multi-spot lights within  $\pm 20\%$ , which was impossible in the prior art.

15 Still further, taking the following measures outstandingly lowers a variation in the beam intensity caused by the reflection and the interference of the beams on the optical component surfaces, thereby making it possible to form the multi-spot lights that allow  
20 the variation in the respective spot energies among the multi-spot lights to be restrained within  $\pm 10\%$ :

Submitting conditions on the multi-layer coat at the time of the beam separation and controlling the manufacturing thereof, or bonding, with an optical  
25 adhesive, the mutually adjacent components out of the optical components such as the polarizer 11e in FIG. 41A, the strip-shaped convex lens array 20e the detail of which is illustrated in FIG. 41C, the pinhole array

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2e, the wavelength plate 3e, and the polarizer 12e.

Incidentally, although, in the above-described embodiments, the explanation has been given concerning the case of forming the 64 or 16 multi-spot  
5 lights, the present invention is not limited thereto and is effective in the case of  $N = 2$ , i.e., the case of obtaining 8 or more of the multi-spot lights.

Next, referring to FIG. 45, the explanation will be given below concerning an embodiment where the  
10 beams obtained by the multi-spot light forming methods explained so far are applied to the fluorescent light detection, especially to the DNA inspection using the fluorescent light detection.

In FIG. 45, numerals 8001, 8002, 8003 ... ..  
15 denote laser light-sources, and include 8 lasers in total that are not illustrated though. The beams emitted from the respective laser light-sources are directed into one direction in parallel to each other with a pitch of about 1.6 mm by mirrors 8011, 8012,  
20 8013 ... .. that are several mm in size. The 8 beams are obtained by the above-described method from the polarization component 12e as if the 64 multi-spot lights were emitted from the mask surface 3e with the 0.4 mm pitch. The 64 multi-spot lights pass through an  
25 image-forming lens 61e, a mirror 63e, a wavelength selection beam splitter 70e, then being illuminated onto an inspection plane 51e of a DNA chip 5e through an objective lens 60e.

On the surface of the DNA chip, there has been performed in advance the probing of a DNA determined for each of predetermined positions. Meanwhile, a target DNA obtained by adding a fluorescent material to a DNA formed by refining and amplifying a living body is poured onto the above-described probe DNA. Then, if the nucleotide sequence of the probe DNA and that of the target DNA correspond to each other, they are hybridized with each other. As a result, the fluorescent material-added target DNA is coupled with the probe DNA.

There exist various types of fluorescent materials to be added to the target DNA. The fluorescent material in the present embodiment absorbs the He-Ne laser light at the wavelength of 633 nm and emits the fluorescent light at the wavelength of 670 nm. The fluorescent light is received by the objective lens 60e, and the transmittance light is reflected by the wavelength selection beam splitter 70e, thereby being guided into a detecting optical system. The wavelength selection beam splitter 70e is a multi-layer coat beam splitter for permitting the 633 nm light to pass through and causing the 670 nm light to be reflected.

The fluorescent light reflected at the beam splitter 70e is reflected at a mirror 71e, then passing through an image-forming lens 62e. The image-forming lens 62e is configured as a telecentric optical system, so that primary light-rays of the fluorescent lights,



15           The detectors 91, 92, which are multi-channel  
photomultiplier tubes, have 32 light-receiving  
apertures each. Spot images of the fluorescent lights  
are separated into two by a right-angled mirror 76e  
located in front of the photomultiplier tubes 91, 92,  
20 then being image-formed at substantially the positions  
of the light-receiving apertures of the photomultiplier  
tubes. There exist pinhole arrays in front of the  
light-receiving apertures of the photomultiplier tubes.  
The pinhole arrays have apertures and a pitch that are  
25 substantially equal to the dimensions of spot images  
that are image-formed by the DNA chip-irradiating 2  $\mu$ m-  
diameter and 20  $\mu$ m-pitch excitation multi-spot lights  
with a magnification M of the objective lens and the



arrangement direction.

FIG. 46 is a diagram for illustrating an embodiment in the present invention. The parallel beams 8e emitted from the one laser light-source or the plurality of laser light-sources are the circularly polarized lights or the linearly polarized lights polarized at 45 degrees toward the paper surface. One beam among the plurality of parallel beams 8e is launched into the one lens 21e within the array 20e of the strip-shaped convex lenses and becomes the converged beam, then being launched into a birefringent material such as calcite. A polarized component of an ordinary ray thereof travels straight without being altered, and a polarized component of an extraordinary ray thereof is refracted. A thickness of this polarization component is set so that a spacing between the ordinary ray and the extraordinary ray at the time of having passed through the polarization component becomes equal to one-half of the arrangement pitch P of the plurality of N incident beam lights 8e. As a result, after having passed through the polarizer 11e', 2N converged beams travel with a pitch of  $P/2$  in parallel to each other.

In the respective beams, the polarized directions in their linearly polarized states differ from each other alternately by 90 degrees. The respective beams are caused to pass through the quarter wave plate 3e having the optical axis that forms 45



degrees toward the polarized directions, or a half wave  
plate 3e having an optical axis that forms 22.5 degrees  
toward the polarized directions, thereby obtaining  
circularly polarized lights or linearly polarized  
5 lights having polarized directions polarized at  $\pm 45$   
degrees toward the paper surface. These 2N beams are  
launched into a polarizer 12e'. The polarizer 12e' is  
a calcite the thickness of which is one-half of that of  
11e'. The beams have passed through the polarizer  
10 12e', which obtains 4N converged beams with a pitch of  
P/4.

These lights are caused to pass through a  
wavelength plate 3e' that is the same as the above-  
explained wavelength plate, thereby becoming circularly  
15 polarized lights or linearly polarized lights polarized  
at 45 degrees toward the paper surface. These multi  
beams are launched into a plate 2e including a multi-  
aperture array that has a pitch and an aperture  
diameter. Here, the pitch is equal to the pitch of the  
20 multi beams, and the aperture diameter is substantially  
equal to a converged diameter determined by the lens  
21e and the diameter of the beams launched into the  
lens. This makes it possible to obtain noise-free  
multi-spot lights including only the lights having  
25 passed through the apertures.

FIG. 47 is an embodiment-illustrating diagram  
for indicating a multi-spot light forming method used  
for the confocal point detection in the present

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invention. In FIG. 47, the same reference numeral as that in FIGS. 41A to 41D denotes the same component. The beams emitted from 1e or the plurality of laser light-sources are formed into the 8 parallel beams, then being formed into the 16 parallel beams by the 2nd beam-doubling prism 4e. The 16 parallel beams are formed into the converged beams by the convex lens array 20e.

Then, the 1st multi-spot lights-doubling prisms 11e, 12e form the 64 multi-spot lights. At this time, the 64 multi-spot lights are obtained as if the 64 multi-spot lights were emitted effectively from the apertures of the mask 3e by the operation of the 1st multi-spot lights-doubling prism 12e. A quarter wave plate 3e' is attached to a light-emitting surface of the multi-spot lights-doubling prism 12e. Thus, the 64 multi-spot lights become circularly polarized lights, then being launched into a polarization component 11e' composed of the calcite. The polarizer 11e', a quarter wave plate 3e'', a polarizer 12e'', a quarter wave plate 3e''', a polarizer 13e', and a quarter wave plate 3e'''' are based on the same beam 2-splitting method as that explained in FIG. 46. Thus, in the polarizers 11e', 12e', and 13e', the thickness of each calcite becomes thinner just as  $t$ ,  $1/2t$ , and  $1/4t$ . Also, the direction in which the beams are going to split is the  $y$  direction. Accordingly, the 64 spot lights are generated in the  $x$  direction by the 1st multi-spot lights-doubling

prisms 11e, 12e, and 8 spot lights are generated in the y direction by the beam 2-splitting method using the calcite. Consequently, as illustrated in FIG. 48,  $64 \times 8$  spot lights are generated in total.

5               FIG. 49 indicates a confocal point detecting method using the above-described  $64 \times 8$  multi-spot lights. In FIG. 49, the same reference numeral as that in the other drawings denotes the same component. The 8 beams emitted from a laser light-source and formed by  
10 an 8-beam optical system 800 are launched into the  $64 \times 8$  multi-spot light-forming optical system 100e illustrated in FIG. 47. Then, in accordance with the above-described method, the  $64 \times 8$  multi-spot lights illustrated in FIG. 48 are being emitted effectively from  
15 the mask surface 3e.

These multi-spot lights are reduced in size and are projected onto a surface of a target 5e' to be measured, using a half mirror 70e', the image-forming lens 61e, and the objective lens 60e. The projected  
20 multi-spot lights are reflected on the surface to be measured, and pass through the objective lens 60e, the image-forming lens 61e, and the half mirror 70e', then being image-formed onto a surface of a  $64 \times 8$ -pinhole mask 901' located directly in front of an image-pickup  
25 surface of a detector 90'. The lights having passed through the pinholes reach the image-pickup surface of the detector. If the surface of the to-be-measured target coincides with an image-forming plane of the

projected multi-spot lights, the reflected light are  
image-formed on the pinholes of the pinhole mask,  
thereby making it possible to obtain the strong  
detected values. If the surface of the to-be-measured  
5 target does not coincide with the image-forming plane,  
out-of-focused spread lights reach the pinholes of the  
pinhole mask 901', and accordingly the intensities of  
the lights having passed through the pinholes become  
lower. Consequently, by executing the above-described  
10 detection with the to-be-measured target being  
displaced in an up-and-down direction by z-stage 503'  
shown in Fig. 49, it becomes possible to obtain the  
heights at the positions of the respective spots from  
their z direction values from which the detection  
15 maximum intensities at the respective positions can be  
obtained.

In the above-explained confocal point detect-  
ing method in FIG. 49, when the target to be measured  
is fixed in the x, y directions, if, for example, the  
20 spot diameter is 1  $\mu\text{m}$  and the spot spacing is 8  $\mu\text{m}$  on  
the surface to be measured, only information about the  
64 $\times$ 8 points with a 8  $\mu\text{m}$  spacing can be obtained.  
Accordingly, it is required to detect the confocal  
point images at the other positions in sequence by  
25 displacing xy stage 501', 502'. FIG. 50 is an  
embodiment-illustrating diagram in the present inven-  
tion, where there is indicated a method of obtaining  
the 2-dimensional confocal point images. The driving

directions  $x'$ ,  $y'$  of the  $xy$  stage in FIG. 49 and the arrangement directions of the multi-spot lights to be projected onto the to-be-measured target are shifted slightly to each other as illustrated in FIG. 50. In  
5 the case of the  $64 \times 8$  multi-spot lights, an angle  $\theta$  formed between these two directions is set so that  $\tan \theta$  becomes equal to  $1/8$ . On account of this shifting, scanning the stage in the  $y'$  direction results in irradiating empty positions between a spot 011 and a  
10 spot 021 with 7 spots, i.e., 012, 013, ... .., 018. This means that the entire surface scanning is performed all over the  $1 \mu\text{m}$  spots with a  $1 \mu\text{m}$  spacing placed therebetween.

The use of the above-described method  
15 irradiates the to-be-measured target with the multi-spot lights having high light intensities, and accordingly it becomes possible to detect the  $64 \times 8$  spot positions in a short time. Thus, one scanning toward the samples is performed in the  $y'$  direction at a high-  
20 speed by the  $y$  stage and after the scanning, the stage is displaced slightly in the  $z$  direction, and the scanning is performed again. This operation is repeated, thereby detecting confocal point signals at the respective heights in sequence at a high-speed. In  
25 this way, a height at which the intensity is a maximum at each point is determined from the 2-dimensional intensity data at each point obtained at each height  $z$ . This makes it possible to execute the height measure-

ment by the high-speed confocal point detecting method. Incidentally, setting the width of the above-described scanning to be equal to  $k$  times the pitch  $p$  of the multi-spot lights results in obtaining the 2-  
5 dimensional height information where the pixel number in the  $x$  direction is equal to  $64 \times 8 = 512$  and the pixel number in the  $y$  direction is equal to  $8k$ .

Next, the explanation will be given below concerning another embodiment for forming the multi  
10 spots.

Here, taking the DNA probe array as an example, the explanation will be given concerning an apparatus and a method for measuring an intensity distribution of the fluorescent lights. The fluo-  
15 rescent lights are emitted from the fluorescent markers for marking the target DNAs trapped in the state of being hybridized with the DNA probes fixed onto the large number of microscopic reaction areas on the DNA probe array. Additionally, it is needless to say that  
20 the present invention is not limited to the inspection of the DNA, but is similarly applicable to the inspection and analysis of the other living body samples such as a fluorescent material-marked RNA, oligonucleotide and protein.

25 The DNA probe array to be detected is formed in the following way, for example: First, a washed glass substrate (such as a slide glass) is treated using a silane coupling agent (aminopropyltriethoxy-

silane), thereby introducing an amino group onto the surface. Next, an aldehyde group-introduced oligonucleotide is dropped on microscopic areas so as to be reacted, thereby fixing the oligonucleotide on the

5 glass substrate. A fluorescent marker-attached DNA is prepared independently as a specimen, i.e., a target to be inspected. Then, the fluorescent marker-attached DNA is spotted onto the oligonucleotide-fixed areas on the substrate so as to be trapped on the substrate by

10 being hybridized therewith, thereby preparing the array to be measured. Incidentally, in order to fix the oligonucleotide on the glass substrate, the following methods are allowable: The surface treatment of the slide glass is performed using poly positive ions such

15 as polylysine, thereby causing the electrostatic bond with electric charges of the DNA. Besides, the oligonucleotide is synthesized directly on the glass substrate, using a photolithography technique and a solid phase synthesizing technique. Being not limited

20 to the above-described forming method, the DNA probe array can be formed by various well-known methods, and is measurable in much the same way.

FIG. 51 is a schematic diagram for illustrating the entire configuration of one embodiment of a

25 fluorescent marker distribution measuring apparatus (DNA probe array measuring apparatus) according to the present invention. The apparatus includes a main body of the DNA probe array measuring apparatus, a data

processing/controlling unit 19f, and a monitor 21f. A substrate (DNA probe array) 2f is fixed on a XYZ driving unit 20f. A light from a light-source 10f of laser or the like is caused to pass through a multi  
5 light-source distribution unit 11f, thereby being emitted as a plurality of lights (100a, 100b, ...) that are located on straight lines equally and uniformly. These lights are collimated by a lens 12f, and are reflected downward by a dichroic mirror 13f, and are  
10 converged by an objective lens 14f, finally forming excitation spots (101a, 101b, ...) onto the substrate 2f. In the drawing, although only 2 of the lights and 2 of the excitation spots are illustrated for concern with the paper space, 64 excitation spots are formed  
15 actually. The larger the number of the excitation spots is made, just like 16, 32, 64, ..., the more effective it becomes to implement the high-speed and high-sensitivity characteristics. Namely, if the total detecting time is the same, the larger the number of  
20 the spots becomes, the more likely it is to be able to lengthen the irradiating time per excitation spot and to be able to enhance the sensitivity. Also, if the irradiating time per excitation spot is the same, the larger the number of the spots becomes, the more likely  
25 it is to be able to shorten the total detecting time. In this case, 64 times speeding-up becomes executable.

Emitted lights (fluorescent lights, scattered light, reflected lights) generated from the excitation



spots on the substrate 2f are converged again by the objective lens 14f, and are caused to pass through the dichroic mirror 13f (a 2-color property mirror for causing wavelength components of the excitation lights to be reflected and permitting longer wavelength components of the fluorescent lights to pass through), thereby extracting the fluorescent light components. Moreover, the fluorescent light wavelength components pass through an interference filter 15f deigned to permit the fluorescent light wavelength components to pass through. In addition, the fluorescent light components are converged by a lens 16f, thereby causing images of the excitation spots (101a, 101b, ...) to be image-formed as fluorescent light images (102a, 102b, ...).

A multi-detection unit 17f substantially simultaneously detects fluorescent light intensities generated from the plurality of excitation spots. Light signals from the multi-detection unit 17f are converted into numerical values by an A/D conversion unit 18f in harmony with a timing from the data processing/controlling unit 19f, then undergoing the processing such as various arithmetic operations by the data processing/controlling unit 19f. Also, the data processing/controlling unit 19f controls displacement of the XYZ driving unit 20f, and constructs a 2-dimensional distribution of the fluorescent light intensities on the substrate 2f in combination with the

XY driving information, then displaying the 2-dimensional distribution on the monitor 21f. This makes it possible to detect the fluorescent light intensity distribution on a predetermined area surface of the substrate 2f, the total fluorescent light intensities of the plurality of microscopic areas provided on the substrate 2f, and so on. Accordingly, it becomes possible to calculate a quantity of the specimen DNAs coupled with the fixed oligonucleotide.

10                Here, on the surface of the substrate 2f, the excitation spots are set to be 2  $\mu\text{m}$  in diameter and the spacing between the excitation spots is set to be about 20  $\mu\text{m}$ . It is desirable to make an adjustment so that the size of the excitation spots becomes equal to 1/an integer-th of the spacing between the excitation spots. In the case of forming the 64 excitation spots, the spacing between both ends of all the spots becomes equal to 1.26 mm. Taking this into consideration, as the objective lens, the one satisfying the following  
15 conditions has been manufactured and used: The field-of-view 1.3 mm, the numerical aperture  $\text{NA} = 0.70$  (in order to enhance a fluorescent convergence efficiency), and (taking into consideration the case where the irradiation is performed from the back surface of the glass) a distance between the lens tip and the sample  
20 position 1.3 mm.  
25

              In order to measure the fluorescent lights all over the entire surface of an attention-paid area

of the substrate 2f, the XYZ driving unit 20f is displaced in the following way: FIG. 65 is an explanatory diagram for explaining an example of the displacement of the XYZ driving unit 20f. The arrangement direction of the excitation spots is taken as an x axis. As illustrated in the drawing, the excitation spots S0, S1, S2, ..., S63 are located in their initial positions in such a manner that the position of the excitation spot S0 becomes (0, 0), i.e., (x, y) = (0, 0). An area 700 is the attention-paid area to be measured which includes the oligonucleotide-fixed areas on the DNA probe array. In order to scan the entire surface of the area 700, the stage is displaced in a 2-dimensional manner. In the drawing, an arrow illustrates an orbit along which the excitation spot S0 is moved relatively to the area 700.

First, all the width of the area 700 is scanned in the y direction. Then, the y positions are returned back to 0, and the spots are shifted by the amount of the excitation spot diameter (in the present example, 2  $\mu\text{m}$ ) in the x direction. All the width of the area 700 is scanned again. This operation is repeated a plurality of times by the time the excitation spot S0 reaches the first position of S1. In the drawing, for simplicity, the scale-reduced illustration is given in such a scale that the spacing between the excitation spots is equivalent to 8  $\mu\text{m}$ , and there is illustrated a manner where the positions in the x

direction are changed 4 times so as to scan all the width in the y direction. Incidentally, in the case where the excitation spots are equal to 2  $\mu\text{m}$  in diameter and the spacing between the excitation spots is equal to 20  $\mu\text{m}$ , it turns out that the above-described operation has been repeated 10 times. This makes it possible to detect the entire surface of the area the width of which is  $x = 0$  to 1280  $\mu\text{m}$ . The next operation is as follows: The y positions are returned back to 0 and the spots are shifted by 1260  $\mu\text{m}$  in the x direction, thereby displacing the spots to an area that is adjacent to the area where the scanning has been finished using the 64 excitation spots. Namely, the spot S0 is displaced to the position corresponding to S64, and the above-described operation is repeated. This is repeated, thereby scanning the entire surface of the area 700.

FIGS. 66A, 66B are diagrams for illustrating displacement curves of x, y axes of the XYZ driving unit 20f. FIG. 66A illustrates a displacement state of the XYZ driving unit in the x direction, and FIG. 66B illustrates a displacement state of the XYZ driving unit in the y direction. As is illustrated in the drawing, the operation of the present driving unit is characterized by repeating at least 2 types of displacement patterns in the x direction, i.e., the arrangement direction of the excitation spots, thereby scanning the entire surface of the to-be-measured area.

Here, the 2 types of displacement patterns are step-like displacements with a microscopic width of  $2\text{ }\mu\text{m}$  at  $n$  times (in the present example, 10 times) and a subsequent step-like displacement with a longer width at one time. This characteristic results from the existence of a limit to the objective lens' field-of-view size and the existence of a restriction to the spacing between the plurality of excitation spots. If the spacing between the excitation spots is made equal to a width obtained by substantially equally dividing the entire surface of the to-be-measured area by 64, it becomes unnecessary to employ the displacement method as described above. Namely, in the  $x$  direction, it is all right to repeat the step-like displacements with the microscopic width of the excitation spot diameter. In this case, however, the spacing between the excitation spots at both ends gets large, becoming substantially equal to a width of the entire surface of the to-be-measured area. This width exceeds the field-of-view width of a common objective lens (i.e., an objective lens being able to form the excitation spots of about  $2\text{ }\mu\text{m}$ ), thereby making it difficult to execute the measurement. If there should exist an objective lens having a sufficiently large field-of-view, the numerical aperture will become smaller or such an objective lens will be an extremely expensive one, either of which is unrealistic. Namely, the scanning method illustrated in FIGS. 65, 66A, and 66B is a

method suitable for scanning the entire surface of the to-be-measured area with the use of the plurality of excitation spots.

Also, as timings of the data collection at  
5 the time of the scanning, there exist 2 timings, i.e., the timing at the time of the displacement in the x direction and the timing at the time of the displacement in the y direction. In principle, either of the timings makes it possible to obtain the information on  
10 the entire surface. However, it is advantageous to collect the data in harmony with the displacement in the y direction in particular. This is due to the following reasons: A stroke of the displacement at one time is longer in the y direction, and accordingly the  
15 connection with the adjacent pixel is more clear, and thus it is easy and secure to reconstruct an image from the measured data.

Next, the explanation will be given below concerning the configuration of the multi light-source  
20 distribution unit 11f. When the size of the excitation spots is 2  $\mu\text{m}$  in diameter and the spacing between the excitation spots is 20  $\mu\text{m}$ , at a light-emitting end of the multi light-source distribution unit 11f, the following is required: Assuming that a coupled  
25 magnification including the objective lens is 20 times, the lights need to be emitted from 64 portions under the condition that, at the light-emitting end, the size of the light spots is 40  $\mu\text{m}$  in diameter and the spacing

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between the excitation spots is  $400\text{ }\mu\text{m}$ . Since it is usually impossible to arrange the light-sources with the spacing of  $400\text{ }\mu\text{m}$ , the following configurations have been constructed in the present invention. These 5 configurations will be explained below, using FIGS. 52 to 57, and FIGS. 67 to 69.

FIG. 52 is a diagram for illustrating the configuration of an embodiment of a multi light-source distribution unit 11f for splitting lights emitted from 10 n laser light-sources (2 of them are illustrated in the drawing) into  $4n$  lights. Lights emitted from laser light-sources (10a, 10b) are each converged by lenses (200a, 200b), thereby being introduced into optical fibers (201a, 201b). Each optical fiber has 2 branch- 15 ing portions halfway, by which each of the converged lights is split into 4 lights. Accordingly, the excitation lights can be split just like 100a1, 100a2, ... 100b4. Incidentally, as the branching portion, it is possible to select not only the 2-branching type as 20 illustrated in the drawing but also the other various types such as a 4-branching type. Also, in such a branching, there is employed a fiber that allows a light to be split equally. For example, light-emitting ends of optical fibers the core diameter of which is 25 equal to about  $40\text{ }\mu\text{m}$  are arranged with the spacing of  $400\text{ }\mu\text{m}$  on a straight line, then projecting the lights in a 1/20th scale-reduced state. This makes it possible to implement the configuration in the present

embodiment.

FIG. 53 is a diagram for illustrating the configuration of another embodiment of the multi light-source distribution unit 11f for simply arranging  
5 lights emitted from m laser light-sources (4 of them are illustrated in the drawing) with the spacing of 400  $\mu\text{m}$  on a straight line. The case  $m = 4$  is illustrated in the drawing. Lights emitted from the laser light-sources (10a, 10b, 10c, 10d) are each converged by  
10 lenses (200a, 200b, 200c, 200d), thereby being introduced into optical fibers (202a, 202b, 202c, 202d). Light-emitting ends of the optical fibers are arranged with the spacing of 400  $\mu\text{m}$  on a straight line. This makes it possible to implement the configuration in the  
15 present embodiment concerning the lights (100a, 100b, 100c, 100d) regardless of the size of the laser light-sources with the spacing of 400  $\mu\text{m}$ .

FIG. 54 is a diagram for illustrating the configuration of another embodiment of the multi light-source distribution unit 11f utilizing the birefringent  
20 material (for example, calcite, and the other materials are also usable). As illustrated in the drawing, birefringent materials 300f, 302f such as calcite and quarter wave plates 301f, 303f are combined with each  
25 other in a sequence of, from the laser light-source side, the birefringent material 300f, the quarter wave plate 301f, the quarter wave plate 302f, and the quarter wave plate 303f, thereby forming a splitting



component 310f. A laser light emitted from a laser light-source 10a is launched into the above-described component through a lens 200a. At this time, the polarization surface of the laser light has been

5 inclined by 45 degrees, or the laser light has been converted into a circularly polarized light by being caused to pass through a quarter wave plate on the way. The laser light launched into the birefringent material 300f is double-refracted, thereby being separated into

10 an ordinary ray and an extraordinary ray. A length of the birefringent material 300f is adjusted so that the two rays are away from each other by a distance  $w_1$ . These lights are converted into circularly polarized lights by being caused to pass through the quarter wave

15 plate 301f further, then being launched into the birefringent material 302f. Each of the 2 lights into which the light has been split is further split into 2 lights similarly. A length of the birefringent material 302f is adjusted so that the distance between

20 the 2 lights becomes equal to  $w_2$ . Making the adjustment so that  $w_1$  becomes 2 times as long as  $w_2$  allows the laser light to be split with an equal spacing. Incidentally, in order to let  $w_2 = 400 \mu\text{m}$ , it is all right to set the lengths (in the optical axis direc-

25 tion) of the birefringent materials 300f and 302f to be about 8 mm and about 4 mm, respectively (in the case where the birefringent materials are calcite and the crystalline axes form 45 degrees toward the optical

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axes). Additionally, the lengths of the respective birefringent materials are varied, depending on the kind of the materials, the wavelength, and inclinations of the crystalline axes. When the directions of the  
5 crystalline axes are identical to each other, as described earlier, the length of the birefringent material is set to be 1/2th of that of the birefringent material at the preceding stage.

This configuration allows the 1 laser light  
10 to be split into the 4 laser lights equally and with substantially the same intensity, and permits the light-emitting ends to be located on a straight line. Thus, locating 16 laser lights with a spacing of  $0.4 \times 4 = 1.6$  mm allows 64 light spots to be located with a  
15 spacing of 0.4 mm on a straight line.. Incidentally, setting a focal point position of the lens 200a to be the position of pinholes 304f makes it possible to eliminate extra lights such as scattered lights. Accordingly, it becomes possible to make beautiful the  
20 configuration of the excitation spots on the substrate 2f, thereby permitting the resolution to be enhanced. Furthermore, the focal point position can be utilized as one focal point position for measuring the confocal point.

25 Also, although the splitting component 310f in the drawing has the configuration of executing the splitting with the 2 stages, it is also possible to employ 3 stages, 4 stages, and 6 stages. In these

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cases, it turns out that one laser light is split into 8, 16, and 64 lights, respectively, and the number of the laser lights required to obtain the 64 light spots can be made smaller like 8, 4, and 1. For example, in 5 the case of a 6-stage configuration where there are employed the birefringent materials the respective lengths of which are 32, 16, 8, 4, 2, and 1 mm in sequence from the light-incoming side, it is possible to split the 1 laser light into 64 lights with a 10 spacing of 0.1 mm.

FIG. 55 is a diagram for illustrating the configuration of another embodiment of the multi light-source distribution unit 11f. The components mainly includes 4 trapezoid-shaped glass prisms 361, 362, 363, 15 and 364 and a quarter wave plate 367. The glass prisms 361, 362, 363, and 364 are connected to each other as illustrated in the drawing, and connected planes 365, 366 are polarization beam splitters. The respective glass prisms differ slightly in their dimensions. 20 Thus, an incident light in a circularly polarized state is split into 2 lights on the connected plane 365, then becoming proximate to each other after the total reflection. At this time, making the optical path lengths differ from each other allows 2 parallel laser 25 light-fluxes to be obtained. When causing the 2 lights to be launched into the components of the glass prisms 363, 364 again through the quarter wave plate 367, each of the 2 lights is further split into 2 lights by the

same principle as that described above, which allows 4  
light-fluxes to be formed in total. The adjustment of  
heights of the trapezoid-shaped glass prisms makes it  
possible to arbitrarily set a spacing between the  
5 light-fluxes, thereby making it easy to obtain the  
light-fluxes with the spacing of 0.4 mm, i.e., the  
light-fluxes that are exceedingly proximate to each  
other. In the drawing, there is illustrated the case  
where the number of emitted light-fluxes is 32 with  
10 reference to 8 incident light-fluxes. Incidentally, it  
is also allowable to locate a pinhole plate in  
proximity to the quarter wave plate 367.

FIG. 56 is a diagram for illustrating the  
configuration of another embodiment of the multi light-  
15 source distribution unit 11f. By using prisms 500a,  
500b, ... illustrated in the drawing which have a  
polarization beam splitter and a total reflection  
mirror, it becomes possible to split a beam into 2  
beams. The use of the prisms by the number of 8 allows  
20 lights from 8 laser light-sources to be split into 16  
lights. Incidentally, it is also allowable to employ,  
as a portion of the polarization beam splitter, a half  
mirror-shaped beam splitter where the transmittance =  
the reflectance (= an order of 50%).

25 FIG. 57 is a diagram for illustrating the  
configuration of another embodiment of the multi light-  
source distribution unit 11f. The prisms 500a, 500b, ...  
in FIG. 56 are arranged, and further thereon, there is

used a prism 510 formed by accumulating larger-sized prisms in sequence. The half mirror-shaped beam splitters split a laser light. Repeating this at 4 stages allows 1 laser light to be split into 16 laser  
5 lights.

In the multi light-source distribution units of the configurations in FIGS. 56, 57, there exists a limit to a task of making the prism sizes smaller. Although the spacing between the laser lights to be  
10 split is allowable down to an order of 1 mm, it is getting gradually difficult to make the spacing even narrower. On account of this, in such a case, the combined use of the distribution units with the multi light-source distribution unit in FIG. 54 or FIG. 55  
15 permits the laser lights to be split into the light-fluxes with a more microscopic spacing.

In general, like the configuration of an embodiment of the multi light-source distribution unit illustrated in FIG. 67, a plurality of multi light-  
20 source distribution units 550, 551 are combined with each other. This makes it possible to construct another multi light-source distribution unit. Namely, the multi light-source distribution units in FIGS. 52 to 57 and what is more, multi light-source distribution  
25 units up to in FIGS. 68, 69 explained later are combined, thereby making it possible to construct another multi light-source distribution unit. Incidentally, the number of the laser light-sources is

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selected suitably in accordance with the number of the splitting, the optical output of the laser light-source, and the light intensities of the excitation spots needed on the surface of the substrate 2f.

5               FIG. 68 is a diagram for illustrating the configuration of another embodiment of the multi light-source distribution unit 11f. A laser light emitted from a laser light-source 2000 is caused to pass through a lens 2001, and is scanned by a beam scanning  
10 unit 2002 such as a galvanometer mirror and a polygon mirror, then being launched into end surfaces of optical fibers 3001, 3002, ... the end surfaces of which are located at a focal point position of the lens 2001 in an arc-like configuration. The other end surfaces  
15 of the optical fibers 3001, 3002, ... are located with a fixed spacing. This allows the laser lights to be split into a large number of light-fluxes, thereby making it possible to implement the configuration in the present embodiment.

20               FIG. 69 is a diagram for illustrating the configuration of another embodiment of the multi light-source distribution unit 11f. The laser light emitted from the laser light-source 2000 is scanned by a beam scanning unit 2003 such as the galvanometer mirror and  
25 the polygon mirror, and is caused to pass through a lens 2004, then being launched into the end surfaces of the optical fibers 3001, 3002, ... that are arranged in a line. The respective split laser lights can be

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extracted from the other end surfaces of the optical fibers, which makes it possible to implement the configuration in the present embodiment.

Next, referring to FIGS. 58 to 63, the  
5 explanation will be given below concerning the configurations of embodiments of the multi-detection unit 17f. Like the present example, images of the excitation spots, the size of which is 2  $\mu\text{m}$  in diameter and between which the spacing is 20  $\mu\text{m}$ , are image-formed  
10 with an appropriate magnification. A pitch of detectors determines the magnification. The detectors 600, 601, ..., such as photomultiplier tubes and photodiodes, are arranged in agreement with the image-formed positions (FIGS. 58, 59). Otherwise, the detection is  
15 executed using a censor 602 such as a photodiode line censor, a multi-anode type photomultiplier tube, and a CCD line censor (FIGS. 60, 61). In the case of the censor 602, a spacing between the individual detectors can be made narrower in comparison with the spacing in  
20 the methods in FIG. 58 and so on. For example, in the multi-anode type photomultiplier tube, the optical system is adjusted so that a spacing between the individual anodes is substantially equal to 1 mm and 50 times image-forming magnification is accomplished.  
25 Incidentally, through the use of pinholes 610 in FIGS. 58, 59, and 60, the multi-detection unit becomes a confocal point detecting system, thereby enhancing a position resolution and a depth resolution and improv-

ing the accuracy of the fluorescent light measurement.  
In the case of using the photodiode line sensor and the  
CCD line sensor, it is also allowable to cause the  
images to be image-formed at arbitrary positions and to  
5 detect signal intensities of the pixels at the image-  
formed positions. It is also possible to obtain the  
same effect as that of the pinholes by precluding  
signals from the pixels at portions other than the  
excitation light spots. It is also possible to execute  
10 the detection through optical fibers 620, 621 (FIGS.  
62, 63).

FIG. 64 is a diagram for illustrating an  
example of the images detected using the present  
apparatus. The drawing illustrates the fluorescent  
15 light spot images captured on the slide glass. The  
spots coupled with the fluorescent marker-attached DNAs  
by the above-described method are formed on the slide  
glass. The size of the respective spots are about 200  
 $\mu\text{m}$ , and the respective spots are formed on a lattice  
20 with a spacing of about 0.4 mm. The entire area over  
which the spots are formed is about 12 mm long and  
wide, and FIG. 64 indicates a portion thereof. In the  
drawing, the respective spots are increased in their  
specimen concentration in sequence from the above. As  
25 a result, the fluorescent light intensity has been  
detected in such a manner as to be increased in  
sequence from the above to the below.

Next, the explanation will be given below

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concerning the data detection and an image reconstruction (i.e., an operation of the data processing unit). In order to detect the signals over the area of 12 mm square in the unit of 2  $\mu$ m, the area of 12 mm square is  
5 divided into 6000 $\times$ 6000 areas. Let these areas be represented by a reference notation: area (i, j), where i (= 0 - 5999) denotes the positions in the x direction and j (= 0 - 5999) denotes the positions in the y direction. The detection of the fluorescent  
10 light intensities based on the scanning by the irradiation spots is executed in the same way as was explained in FIG. 65. Explaining the scanning performed by the respective irradiation spots (S0, S1, ..., S63), with respect to the scanning by the irradiation spot S0,  
15 area (0, 0) is first irradiated with the irradiation spot S0 and then, following the scanning in the y direction, the irradiation spot scans the areas from area (0, 1) to area (0, 5999). With respect to the scanning by the irradiation spot S1 as well, area (10,  
20 0) is first irradiated with the irradiation spot S1 and then, following the scanning in the y direction, the irradiation spot scans the areas from area (10, 1) to area (10, 5999). The other irradiation spots S3 to S63 perform the scanning in much the same way. With  
25 respect to the scanning by the irradiation spot S63 as well, area (630, 0) is first irradiated with the irradiation spot S63 and then, following the scanning in the y direction, the irradiation spot scans the

areas from area (630, 1) to area (630, 5999).

At that time, the signals from the optical detector corresponding to each spot are sampled every time each spot passes through the areas, then being  
5 A/D-converted. The signals are expressed as  $\text{sig}(k, j)$ , where  $k$  corresponds to a position number (0 - 63) of the spots and  $j$  takes the values of 0 to 5999 at the positions in the  $y$  direction. Namely, the signals from the spot  $S_1$  are expressed as  $\text{sig}(1, j)$ ,  $j = 0 - 5999$ .

10 These signals are stored into image storing memories corresponding to the areas of area  $(i, j)$ . Letting the image storing memories be represented by  $\text{image}(i, j)$  where  $i$  ( $= 0 - 5999$ ) and  $j$  ( $= 0 - 5999$ ) denote the positions in the  $x, y$  directions, respectively, the  
15 signals obtained by the above-described scanning at one time are stored into

$\text{image}(k \times 10 + X, j) = \text{sig}(k, j) (X=0, k=0-63, j=0-5999).$

Incidentally,  $X$  denotes an  $X$  position of the spot  $S_0$  (In this case,  $X = 0$ ).

20 Then, as illustrated in FIG. 65, the  $Y$  position is returned back to 0, and the  $X$  position is displaced by the amount of  $+2\mu\text{m}$  ( $X = 1$ ), then executing the detection in the same way. The signals obtained are stored into

25  $\text{image}(k \times 10 + 1, j) = \text{sig}(k, j) (k=0-63, j=0-5999).$

Repeating this operation ten times causes areas indicated by  $i = 0 - 639$ ,  $j = 0 - 5999$  in  $\text{image}(i, j)$  to be replaced by the measured signals.

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Next, the X position is displaced by + 1260  $\mu\text{m}$  (a position of X = 640), then, similarly, causing the above-described operation to be performed and storing the obtained signals into

5 `image(k $\times$ 10+X,j) = sig(k,j)(X=640-649, k=0-63, j=0-5999).`

This operation causes areas indicated by i = 640 - 1279, j = 0 - 5999 in image (i, j) to be replaced by the measured signals. Moreover, the X position is displaced by +1260  $\mu\text{m}$  (a position of X = 1280), then

10 repeating the above-described measurement. These are repeated, thereby displaying the X position up to a position of X = 5760, and causing the scanning to be performed 10 times further. This measures all the areas indicated by i = 0 - 6399, j = 0 - 5999 in image  
15 (i, j) (Although this area is wider than area (i, j), it is all right to extract a needed area alone). This image (i, j) is then converted into a picture, which is displayed on the monitor or the like.

In the present embodiment, there are provided  
20 the excitation spots and the optical detecting unit corresponding to each spot. There occurs no problem if the intensities of all the excitation spots and a sensitivity characteristic of the optical detecting unit coincide with each other. If not, there appears a  
25 stripe-shaped pattern in the obtained picture. In such a case, there is a need of a mechanism for correcting the sensitivity characteristic for each spot. Thus, in FIG. 51, there is provided an intensity correction

Preparing a specimen having equal fluorescent light intensities (or locating light-sources that emit lights

15 becoming a reference value is detected (the signal intensity values obtained by subtracting the background value). By outputting signal values  $\text{sig}(k, j)$  corrected as

```
sig(k, j) = (sig(k, j) - back(k))corr(k)
```

20 toward the measured signals  $\text{sig}(k, j)$ , it becomes possible to correct a variation in the sensitivity among the spots.

In contrast to the circumstances that the ordinary apparatus uses only 1 fluorescent-light  
excitation irradiation spot, the present invention  
25 allows the substrate to be simultaneously irradiated with the plurality of (64, for example) fluorescent-light excitation irradiation spots. This condition

results in the following effects:

When detecting the entire area in a fixed time-period, it is possible to lengthen the irradiating time per irradiation spot (64 times in the present embodiment). This enhances the fluorescent-light detecting sensitivity. Also, lengthening the irradiating time per irradiation spot also makes it possible to slow the displacement speed of the stage. This enhances the mechanical stability and durability as well. Also, equating the irradiating time per point makes it possible to shorten a time needed to measure the whole of a predetermined region on the substrate. This allows the high-speed characteristic to be implemented. In particular, when enhancing the detection resolution, namely, when making smaller the size of the irradiation spots on the substrate, if a target that has been measured conventionally with a resolution of, for example,  $10\text{ }\mu\text{m}$  is measured with a resolution of 1 to  $2\text{ }\mu\text{m}$ , it is required to measure in sequence the irradiation spot regions that are 100 to 25 times more in number. On the other hand, just like the present invention, by simultaneously irradiating the substrate with the plurality of (64) fluorescent-light excitation irradiation spots so as to execute the fluorescent light detection, it becomes possible to accomplish the high-resolution and high-speed characteristics.

Incidentally, when performing the irradiation with the split laser lights, the intensity of the

individual split laser lights becomes weaker by the amount of being split. The use of a larger output laser apparatus, however, allows this problem to be solved easily.

5           According to the present invention, it becomes possible to measure the DNA probe array and so on with a high-resolution, at a high-speed, and with a high-sensitivity.

FIG. 70 is another embodiment-illustrating  
10 diagram in the present invention. Laser light-sources 211g, 212g, 213g ... emit parallel round beams that are converted from diffused lights emitted from semiconductor lasers having a wavelength of 635 nm. The K light beams emitted from the respective laser light-  
15 sources are launched into multi-beam splitters 22g with a pitch P. The multi-beam splitters 22g are arranged with a pitch P by the number of K, and split each of the incident beams into 2 beams, thereby forming 2K beams with a pitch P/2 and in parallel to each other.  
20 These beams have been formed into elliptically polarized lights, linearly polarized lights, or circularly polarized lights, the elliptically polarized lights having the longer axis in the directions that forms  $\pm 45$  degrees toward y, z axes of the illustrated  
25 coordinate.

The 2K beams are arranged with the pitch P/2, then being launched into a multi lens 231g. The multi lens 231g is pasted on a prism 232g. The beams that

have passed through the multi lens pass through the prism 232g, then being launched into multi pinholes positioned at the boundary with a prism 233g.

At both of the bases of the prisms 232g,  
5 233g, trapezoid-shaped glasses that differ from each  
other slightly in thickness are bonded to each other.  
The bonded plane is formed as a polarization beam split  
plane. As a consequence, the beams having passed  
through the prism 232g are converged into the respec-  
10 tive pinholes of a pinhole array 234g having the 4K  
pinholes with a pitch  $P/4$ . The pinhole array plays a  
role of eliminating a stray light so as to irradiate  
the DNA chip with the multi spots having a less noise,  
the stray light existing in a portion other than the  
15 pinhole unit and becoming the noise.

At the time of passing through the above-described prism 232g, the lights having passed through the polarization beam split plane become P-polarized lights (linearly polarized lights perpendicular to the z axis), and the lights reflected by the beam split plane become S-polarized lights (linearly polarized lights in the z axis). On account of this, the half of the lights having passed through the multi spots are the P-polarized lights, and the remaining half are the S-polarized lights.

Directly behind the pinhole array, there is located a half wave plate or a quarter wave plate. The half wave plate has an optical axis inclined at  $22.5^\circ$

degrees toward the above-described P-polarized and the S-polarized lights. Also, the quarter wave plate has an optical axis inclined at 45 degrees toward the lights. The P-polarized and the S-polarized lights  
5 that have passed through such a wavelength plate after passing through the pinholes become linearly polarized lights inclined at  $\pm 45$  degrees toward the z axis in the case of the half wave plate, and become right-handed and left-handed circularly polarized lights in the case  
10 of the quarter wave plate.

The lights having passed through the above-described wave plate 235g are launched into the above-described trapezoid-pasted prism 233g (Additionally, the difference in thickness between the trapezoid-  
15 shaped prisms is one-half of the thickness of the above-described trapezoid-pasted prism 232g). The pasted plane, i.e., the trapezoid-shaped base of the prism, is formed as the polarization beam split plane. As a consequence, the 4K lights having passed through  
20 the pinhole array and the wavelength plate are increased by 2 times in number even further, eventually having a pitch of  $P/8$  and becoming 8K in number. On account of this, the lights are emitted from the prism 233g as if 8K point light sources (secondary point  
25 light sources) existed at the position of the pinhole array 234g.

The lights from the 8K secondary point light sources formed in this way pass through a lens 24g, a

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mirror 25g, a light amount adjuster 26g, a wavelength selection beam splitter 30g, and a high NA objective lens 3g, then image-forming images of the 8K point light sources onto a detection plane 51g of a DNA chip

5 5g. Incidentally, the light amount adjuster 26g is provided in order to adjust a light amount of the fluorescent-light detection excitation lights depending on to what extent the fluorescent material exists on the detection plane 51g. Namely, if the concentration

10 of the fluorescent material is high all over the entire DNA chip or fluorescent light detection sample, a driving source 260g is driven so that a ND filter 262g of, for example, 10% is inserted into the excitation light optical path. However, changing the intensities of all

15 the excitation light beams in this way, in some cases, results in narrowing the dynamic range of the detection. Consequently, in such a case, it is possible to adjust the intensities of the individual excitation light multi spots using a light modulator array not

20 illustrated.

Also, when it is required to execute the detection in a dynamic range of, for example,  $2^N$ , the following detecting method is also possible: Setting a ND filter 261g to be 100% (i.e., there exists no ND

25 filter) and setting the ND filter 262g to be a transmittance of  $100 \times 2^{N/2} \%$ , the ND filters are exchanged so as to execute a 2-picture detection toward the entire picture. Then, both of the detected

pictures are superimposed, thereby detecting the  
fluorescent light images in the dynamic range of  $2^n$ . In  
this way, the detection of the 2 pictures necessitates  
a time twice as long as the time in the ordinary  
5 method. The present invention using the multi spots,  
however, makes it possible to execute the detection at  
a speed that is more than 5 times higher as compared  
with the speed in the conventional methods.

The 8K point light source images that have  
10 illuminated the detection plane of the DNA chip  
illuminate and excite a target DNA having the fluo-  
rescent material at a tip hybridized with a probe DNA.

FIG. 71 is a diagram for indicating the  
relationship between the multi spots and the details of  
15 an area 50g where the target DNA exists on the glass  
51g of the DNA chip 5. A numeral 505 denotes a cell.  
The cells are arranged in the x, y directions with a  
fixed pitch. In each cell, a desired DNA fragment,  
which is also referred to as the probe DNA, is attached  
20 to the glass for each address in the arrangement of the  
cells. This probe DNA is a DNA that usually includes  
the same nucleotide sequence in each cell, and a  
different DNA is usually probed in a different cell. A  
sample solution of the target DNA obtained by attaching  
25 the fluorescent material to ends of plural types of DNA  
fragments formed by collecting, refining and amplifying  
a target to be inspected, i.e., a specimen of a crea-  
ture, is poured onto the DNA chip prepared in the

above-described manner, thereby causing the hybridization to occur. Namely, a target DNA corresponding to the nucleotide sequence of the probe DNA in each cell is coupled with the probe DNA, namely, is hybridized  
5 therewith. The DNA chip in FIG. 71 has been formed in the above-explained manner.

With reference to the cells 505, the multi spots 2001g, 2002g, 2003g, ... ..., 20Mg, i.e., the pumping lights, are arranged with a pitch that is an  
10 integer multiple of the cell pitch. In the case of FIG. 71, this integer is equal to 2. The beam diameter of the multi spots is equal to substantially  $\Delta$ , and the pitch between the multi spots is equal to  $N\Delta$ . In the case of FIG. 71,  $N = 10$  and the detection is executed  
15 by dividing one cell into  $5 \times 5$  pixels. An object of such a division is as follows: When a foreign substance or the like is attached in a cell, the foreign substance generates a strong fluorescent light, thereby causing an error in the detected data. Accord-  
20 ingly, judging that a fluorescent light exhibiting a detection intensity larger than the strong fluorescent light detection intensity is generated by the foreign substance, an average fluorescent light intensity within the cell is determined by excluding the pixels  
25 where the foreign substance has been attached.

The DNA chip is scanned in the x direction as is indicated by A in FIG. 71, and when the multi spots scan the cells positioned at the right end, the multi

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selection beam splitter 30g. Then, the reflected  
fluorescent lights are guided into a detecting optical  
system. Namely, the reflected fluorescent lights pass  
through a mirror or a wavelength selection beam  
5 splitter, i.e., 32g, 34g, 35g, and 38g, and pass  
through an image-forming lens 33g, then being image-  
formed at light-receiving apertures of multi-channel  
photomultiplier tubes 101g, 102g. At the respective  
light-receiving apertures, there is provided a light-  
10 shielding plate where pinholes are bored, the diameter  
of the pinholes being substantially equal to that of  
the images of the excitation multi-spot lights. Only  
the fluorescent lights that have passed through the  
pinholes are detected, thereby executing the confocal  
15 point detection.

An upper graph in FIG. 73 is a graph obtained  
by enlarging the time axis of the lower graph in FIG.  
72. In the drawing, the time  $t_s$ , which is needed to  
execute one scanning, is seen in more detail. In a  
20 lower graph in FIG. 73, although the transverse axis,  
i.e., the time axis, is identical to the transverse  
axis of the upper graph in FIG. 73, the longitudinal  
axis indicates a timing of a time of the photon  
counting performed by a weak light detector such as the  
25 photomultiplier tube. This graph means that the photon  
counting is performed during a time for which the  
signal level is 1 and the photon counting is not  
performed during a time for which the signal level is

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0. During the one-sectioned time for which the signal level is 1, the signal by the amount of one pixel is detected. After the signal level becomes 0, when the signal level 1 starts next, the next pixel is detected.
- 5 Since there exist  $L_x$  pixels in one scanning in the x direction, the detecting time per pixel becomes equal to  $t_s/L_x$  or less. The time needed to perform the photon counting on the per-pixel basis is required to correspond to the position of the cell in the DNA chip.
- 10 Consequently, the signal for the starting time of the photon counting is created based on a signal from the length-measuring device for measuring the x stage's position.

- If the finishing point-in-time of the per-
- 15 pixel photon counting is determined in accordance with the information from the stage's position length-measuring device, when the stage is not displaced uniformly, the per-pixel photon counting time is varied for each pixel. This requires that the finishing time
- 20 be a time that has elapsed by a fixed time after the starting time. For the purpose of this, pulses of a high-frequency (frequency  $\nu_r$ ) pulse generator is counted. The counting is started from the starting of the photon counting, and the photon counting is
- 25 finished at the time when the pulse number counted reaches a fixed pulse number  $N_r$ . Namely, with reference to whatever pixel, the photon counting is executed during a time of  $N_r/\nu_r$ . A control apparatus

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1g in FIG. 70 is a system for controlling the above-described operation. A reference numeral 12g denotes a PC (personal computer), which performs the control of all the operations. A PC 13g generates a controlling and driving signal of the above-described stage 4g, and fetches the position information of the stage 4g from the length-measuring device.

In the above-described manner, the fluorescent lights from the 64 pixels in the x direction are detected simultaneously and, through the scanning by the stage, in sequence. Photon counting signals  $S_{PM}$  to be obtained from each pixel are detected in the manner as is illustrated in FIG. 74. Namely, focusing an attention on one photomultiplier tube out of the multi-channel photomultiplier tubes, in the case of weak detected fluorescent lights, photomul signals are obtained as photon detection pulse signals the pulse time-width of which is equal to about 30 ns with a low frequency as are illustrated in an upper graph in FIG. 74. Then, pulse signals with a fixed width are generated from the rising edges of the photomul signals. Otherwise, in the case where the photomul signals exceed a fixed signal level  $I_T$  as are illustrated in the upper graph in FIG. 74, the photomul signals are converted by a comparator circuit into fixed level signals just like the signals  $S_{PC}$  illustrated in a lower graph in FIG. 74. The pulse-shaped signals formed in this manner are counted using a

counter. This allows the detected photon number to be determined within the detecting time  $t_s/L_x - \Delta t = Nr/\nu r$ .

FIGS. 75A to 75D illustrate the case where the detected fluorescent light intensities are high.

5 The drawings illustrate the photomul detection signals  $S_{PM}$  and the signals  $S_{PC}$  after being compared. The photon pulses are detected with a high frequency and accordingly, in some places, 2 or more of the pulses have been detected in a state of being superimposed on  
10 each other. On account of this, among the signals after being compared, there appear signals having larger width.  $S_{PC}'$  is a diagram resulting from enlarging a time of the signals  $S_{PC}$  between a point-in-time A and a point-in-time B. If the detection signals have been  
15 detected in the state of being superimposed between a signal and a signal subsequent thereto, the pulse-widths of the signals after being compared are increased. As a result, calculating simply the pulse number of the signals after being compared results in  
20 an increase in the error. Accordingly, as illustrated in a graph in FIG. 75D, constant high-frequency pulse signals are counted with  $S_{PC}'$  employed as the gate. The use of the counted signals  $S_{PWC}$  thus obtained makes it possible to determine the photon number more precisely  
25 than counting the pulses of the  $S_{PC}$  signals. Moreover, by correcting the counted value of the  $S_{PWC}$  signals with a downward-convex univalent function  $F(S_{PWC})$ , it becomes possible to execute even more precise photon counting.

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The above-described method, however, is insufficient to implement the even broader dynamic range requested in the DNA inspection. Namely, in the DNA inspection, the dynamic range of several photon  
5 pulses to one hundred and tens of thousands of photon pulses is requested within the time needed to detect one pixel. Nevertheless, since the width of the photon detection pulses is equal to tens of ns, when trying to implement the entire dynamic range by the photon count-  
10 ing, tens of ns  $\times$  one hundred and tens of thousands = several m sec become required as the per-pixel detecting time.

Accordingly, even if the number of the multi-spot excitation lights is equal to 64, when the number  
15 of the pixels is  $6000 \times 6000$ , it turns out that it takes 30 minutes or so to execute the detection.

FIG. 76 illustrates an embodiment for solving this problem. The output signal of the multi-channel photomultiplier tube 101g is inputted into an analogue  
20 switch 1A. A PC 10g connects an initial condition of the analogue switch with B, i.e., a photon counting circuit 1B. This means that, when executing the fluorescent light detection on the per-pixel basis, the photon counting is executed first. FIGS. 77A, 77B,  
25 which illustrate the time-elapsing change of the photon counting signals, correspond to the case where the fluorescent lights are exceedingly weak and the fluorescent light intensities are detected by the photon

counting detection.

FIG. 77A illustrates the pulse signals  $S_{pc}$  formed by binarizing the photon pulse signals with the use of the comparator, the photon pulse signals having been detected by the photomultiplier tubes. FIG. 77B illustrates a counted signal  $NS_{pc}$  obtained by counting the pulse number of the pulse signals in FIG. 77A. If the count signal  $NS_{pc}$  does not exceed a determined threshold value  $N_0$  at a point-in-time at which a time  $\alpha$  ( $t_s/L_x$ ) has elapsed after the starting of the counting, the analogue switch continues the photon counting in the present state that will remain unchanged, then executing the photon counting during the per-pixel fluorescent light detecting time  $t_s/L_x - \Delta t$ . With respect to  $\alpha$  in the time  $\alpha(t_s/L_x)$  at which this judgement should be made after the starting of the counting, it is all right to assume:  $\alpha$  to be substantially 0.01 to 0.3. Namely, this time is satisfying enough if it is of an order of a time during which the photon counting pulses are at the maximum and approximately 10 or more pulses are detected. The above-described phrase "at the maximum" means the case where the pulse signals have been generated at the highest ultra-density within this time.

FIGS. 77C to 77E illustrate the case where the fluorescent light intensities become higher and executing the photon counting causes inconveniences to occur, namely, the case where 2 or more of the photon

counting pulses are superimposed on each other at the same time or partly. In this case, the after-comparator pulse signals  $S_{pc}$ , when formed by comparing the partly superimposed pulses, are increased in their widths as illustrated in FIG. 77C. FIG. 77D illustrates a counted signal  $NS_{pc}$  of the pulse signals in FIG. 77C. The counted signal  $NS_{pc}$  exceeds the threshold value  $N_0$  at the point-in-time  $\alpha(t_s/L_x)$ , which shows that the fluorescent light detection by the photon counting is not suitable for the fluorescent light intensities at the point-in-time. Thus, at this point-in-time, the analogue switch in FIG. 76C is connected to C so as to integrate the photomul signals in an analogue manner by an integrating circuit 1C. In this way, by judging, on the per-pixel basis, whether the fluorescent light detection should be executed by the photon counting or the analogue integration, the detection is executed in such a manner that the optimum method is selected for each detection.

Incidentally, although not illustrated, if the fluorescent light intensities become higher than those in the state in FIG. 77C, the pulses in FIG. 77C are connected with each other and as a result, the pulse-counted value become equal to several or 0. At this time, as illustrated in FIG. 75D, employing the signals  $S_{pwc}$  for counting the signals  $S_{pc}$  with the use of the rectangle-shaped high-frequency pulses with the constant frequency, a pulse-counted signal  $NS_{pwc}$  thereof

5 detection to be executed without a mistake even in the  
case of the detection of the even higher fluorescent  
light intensities.

Once the switching to the analogue detection has been performed, the circuit 1C starts the analogue integration with the switching point-in-time employed as the starting point-in-time. As illustrated in FIG. 78A, setting a target pixel detection starting point-in-time to be the starting point-in-time (this point-in-time is assumed to be 0), the circuit 1C starts the integration from the point-in-time  $\alpha(t_s/L_x)$ . Then, as is the case with the photon counting, the circuit 1C continues the integration until the point-in-time  $t_s/L_x - \Delta t$ , and A/D-converts the integrated value at this point-in-time. The A/D-converted digital information IC01 is transferred to a circuit 100g. In the case where the fluorescent light intensities are weak, the photon counting is continued, and the photon counting signals ranging from the point-in-time 0 to the point-in-time  $t_s/L_x - \Delta t$  are counted. The counted digital information IB01 is also transferred to the circuit 100g. The results of the photon counting digital information and the analogue integration digital information, which have been transferred to the circuit 100g

in the parallel manner as described above from the respective channels of the multi-channel photo-multiplier tube 101g, are transferred to the PC 11g, i.e., the personal computer. As having been described previously, it was recognized at the point-in-time  $\alpha$  ( $t_s/L_x$ ) whether the detection executed in each channel has been executed by the photon counting or the analogue integration. Consequently, the PC 11g selects and employs the method suitable for the detection for each channel. Namely, the selection signals therefor are simultaneously transferred to the information IC01, IC02, ... in Fig. 76.

Incidentally, in the above-described embodiment, it was judged at the point-in-time  $\alpha$  ( $t_s/L_x$ ) which method should be selected of the photon counting and the analogue integration. However, it is also allowable to execute the detection from the beginning by both of the methods simultaneously and to perform the selection after the time  $t_s/L_x - \Delta t$  has elapsed.

FIG. 78A indicates a variation in the integration signal when the analogue integration detection is executed. Between the point-in-time  $\alpha$  ( $t_s/L_x$ ) at which the analogue integration detection was started and the point-in-time  $t_s/L_x - \Delta t$ , the integrated value has been increasing with an inclination proportional to the magnitudes of the detected fluorescent light intensities. Then, the integrated value at the point-in-time  $t_s/L_x - \Delta t$  undergoes the sample holding,

then being A/D-converted. The analogue detection as described above makes it possible to capture the variation in the signal intensities over a dynamic range of approximately 2 order.

5               Next, referring to FIG. 78B, the explanation will be given below concerning a method of making the detection executable in the range of the detection of the even higher fluorescent light intensities. In this case, since the integrated value had been saturated  
10 before the analogue integration detection reached the point-in-time  $t_s/L_x - \Delta t$ , the detection at this point-in-time is impossible: However, using the high-frequency pulse signals  $S_{hc}$  used in FIG. 75D, by counting  $(t_f - \alpha(t_s/L_x))$ , i.e., a time ranging from the  
15 point-in-time  $\alpha(t_s/L_x)$  at which the integration was started to a point-in-time  $t_f$  at which the integration was saturated, the detected intensity  $I$  is determined by the following equation since the integration-saturated value  $I_f$  had been known in advance:

20               
$$I = I_f \cdot ((1-\alpha)t_s/L_x - \Delta t) / (t_f - \alpha(t_s/L_x))$$

In this way, measuring the time from the integration-starting point-in-time to the integration-saturated point-in-time allows the dynamic range to be enlarged by approximately 1 order further.

25               In order to count the saturated point-in-time as described above, it is well enough to add the

following function to the analogue integrating circuit illustrated in FIG. 76. Namely, setting the integration signal of the integrating circuit 1C to be the 1st input and setting a signal level equivalent to the  
5 predetermined integration-saturated value to be the 2nd input, these two inputs are inputted into the comparator circuit. Then, from variations in the outputted signals resulting from the inputs, it can be assumed that the counting has been finished. This  
10 allows the saturated point-in-time to be counted.

As having been explained so far, the fluorescent lights detected by the multi-channel photomultiplier tubes 101g, 102g are detected in the 64 channels in parallel, and are inputted into the circuit  
15 100g in parallel and are converted into the time-sequential signals, then being transferred to the PC 11g. By scanning the x stage in FIG. 70, as illustrated in FIG. 71, the 64 pixels arranged in the y direction are detected simultaneously and, in the x  
20 direction, in sequence. Although not illustrated, the optical length-measuring device is attached to each of the xy stage 4g in FIG. 70, and the length-measuring device measures the displacement quantities of the xy stage. The dimensions  $\Delta x$ ,  $\Delta y$  of the fluorescent light  
25 detected pixels on the DNA chip are each assumed to be, for example, 2  $\mu\text{m}$ . The measurement of each pixel is started from a point-in-time at which the length-measuring device of the x stage measures the displace-

ment of 2  $\mu\text{m}$ .

Hereinafter, the explanation will be given concerning a concrete embodiment of the multi-spot fluorescent light detection or the multi-spot DNA inspection according to the present invention. The scanning operation is performed in the x direction, and the shifting operation by the amount of 1 pixel on the basis of one scanning in the x direction is performed in the y direction, and the x positions are shifted by a large amount every 10 scanings. This is repeated, thereby executing the detection or the inspection in the 2-dimensional manner.

When a stage's position length-measuring signal of the measurement starting is detected by the scanning in the x direction, the photon counting is started first. It is assumed that  $L_x$ , i.e., the detected pixel number in the x direction, is equal to 6400, and the pixel pitch is equal to 2  $\mu\text{m}$ , and  $t_s$ , i.e., the time needed to scan this 2  $\mu\text{m}$ , is equal to 60  $\mu\text{sec}$ . The width of the pulses generated after the photomultiplier tube has received 1 photon is equal to substantially 30 n sec. Consequently, when executing the photon counting during 40  $\mu\text{sec}$  out of the time of 60  $\mu\text{sec}$  during which the spot has passed through one pixel, it becomes possible to count hundreds to thousands of photons using the photon counting method. Toward the lights the intensities of which are higher than this, the pulse signals are connected with each



tion together in a manner of being switched to each other by the above-described method, it becomes

10 explained in FIG. 78B by which the detected intensity  
is determined from the point-in-time at which the  
analogue integration was saturated, it becomes possible  
to enlarge the dynamic range by 1 order further. As a  
consequence, it becomes possible to execute the fluo-  
15 rescent light detection in a short time over a broad  
dynamic range of  $2^{16}$ , i.e., 65536, namely, from the weak  
detected lights of several photons in 40  $\mu$ sec up to the  
strong detected lights attaining to 100000 photons.

intensities include 3 areas, i.e., the photon counting area, the analogue integration area, and an analogue integration-saturated time detecting area, in order to cause the respective boundaries therebetween to be connected with each other smoothly, the calibration is performed in advance using strong detected lights corresponding to the boundary regions.

Although, in the above-described embodiment in FIG. 76, the detection is executed by time-dividing

the photon counting and the analogue integration, it is also possible to use both of them all the time and to select the signal to be employed in correspondence with the intensities of the detected fluorescent lights.

5           The above-described switching between the detecting methods is not limited to the fluorescent light detection, but is generally effective in the case where the per-pixel detecting time  $T_p$  is restricted to a short time. Namely, when  $N_r$ , i.e., the ratio between  
10  $T_p$  and the time-width  $\Delta t$  of the photon detection pulses, is smaller as compared with the requested detection dynamic range  $N_d$ , namely, when

$$T_p / \Delta t = N_r < N_d$$

holds, the detection becomes executable in the broad  
15 range extending from the weak photon counting area up to the strong lights the photon counting of which is impossible.

The scanning by the amount of 6000 pixels is performed in the x direction at a speed of 60  $\mu\text{sec}/1$   
20 pixel and at this time, in the y direction, 64 pixels are detected simultaneously with 10 pixels skipped. Accordingly, at a point-in-time at which the one scanning is over in spending 360 msec, 384000 pixels  
have been detected in the above-described broad dynamic  
25 range. When the one scanning is over, the stage is displaced in the y direction by the amount of 1 pixel,

i.e.,  $2\ \mu\text{m}$ , then detecting the adjacent pixel line similarly. This operation is repeated 10 times, thereby executing the detection of  $640 \times 6000$  pixels. Even if accelerating and decelerating times needed to return  
5 the stage are added, the detection of the pixels in the above-described number is finished in spending 4 to 5 seconds.

Next, the stage is displaced largely in the y direction by the amount of 640 pixels, i.e.,  $2\ \mu\text{m} \times$   
10  $640 = 1.28\ \text{mm}$ , then repeating the above-described operation. Repeating this large displacement 10 times allows  $6400 \times 6000$  pixels to be detected in spending substantially 40 to 50 seconds. Namely, it becomes possible to accomplish the fluorescent light detection  
15 or the weak light detection of the images of  $6000 \times 6000$  pixels the minimum detection intensity of which is of several photons with a resolution of  $2\ \mu\text{m}$ , in the dynamic range of  $2^{16}$ , and within 1 minute.

The above-described embodiment is of the case  
20 where the detecting time requested for each pixel is short. However, in the case where the per-pixel detecting time is larger by 1 order or more and the dynamic range of  $2^{16}$  is required similarly, the method illustrated in FIG. 78B where the integration-saturated  
25 time is determined becomes unnecessary. Also, if the width of the photon detection pulses from the photomultiplier tube can be shortened even further, the photon counting alone or the combination of the photon

counting and the analogue integration detection makes the detection possible.

When trying to apply the DNA inspection, in particular, the DNA inspection using the DNA chip, to a usage such as group checkup where a large number of samples are dealt with, it becomes important to implement an inspecting apparatus having the characteristics of a high-sensitivity, a high-resolution, a broad dynamic range, and a high-speed. This implementation has been made possible by the following method: Using the multi-spot excitation lights or the sheet-shaped excitation beams explained in the previously-mentioned embodiments, the fluorescent lights from a plurality of pixels are detected simultaneously, and the relative positions between the excitation lights and a sample are changed by the scanning so as to detect the fluorescent lights in sequence.

Detecting the 64 ( $M = 64$ ) pixels simultaneously has allowed the  $6000 \times 6000$  pixels to be detected in spending a time less than ( $300 \mu\text{sec}/\text{pixel}$ ), namely, the average pixel detecting time has become shorter than ( $300 \mu\text{sec}/M$ ). As a result, it has become possible to execute the detection in a time less than 3 minutes per sample. This result can respond to and satisfy the needs of the high-speed inspection because, conventionally, it took 10 minutes or more to detect the fluorescent light images of the pixels in the above-described number. Furthermore, by using the photon

counting and the analogue integration together, it becomes possible to execute the detection in a time less than (50  $\mu$ sec/pixel), thereby eventually allowing the detection to be executed within 1 minute.

- 5                   Moreover, in the above-described fluorescent light detection using the multi spots, the diameter of the multi spots on the sample is made smaller, thereby performing the irradiation with the multi-spot excitation lights or the sheet-shaped excitation lights.
- 10   Here, the multi-spot excitation lights include a large number of M microscopic spots the diameter of which is smaller than 3  $\mu$ m and larger than 0.3  $\mu$ m, and the sheet-shaped excitation lights have a focus-achieving width that is smaller than 3  $\mu$ m and larger than 0.3
- 15    $\mu$ m. The fluorescent lights generated by the irradiation from the respective multi spots or the respective sheet-shaped irradiation locations are separated from the excitation lights, then detecting the fluorescent light images emitted from the sample with the use of a
- 20   plurality of weak light detecting components. Signals obtained from the respective detecting components are stored individually. Then, the relative positions between the multi-spot excitation lights or the sheet-shaped excitation lights and the sample are changed,
- 25   thus storing the above-described signals in sequence. This allows the signals to be stored and collected over a desired range on the sample. Then, the fluorescent light picture is constructed from the collected data,

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thereby making it possible to inspect the DNA. The use of the method as described above enhances the resolutions of the detected images. Utilizing the enhanced resolutions makes it possible to detect a to-be-  
5 inspected DNA in a cell as the fluorescent light images and to execute the DNA inspection of the cell in the following way: For example, employing, as a sample, a desired target DNA to which a fluorescent light material has been added, the fluorescent light  
10 material-added target DNA is hybridized with the corresponding DNA among 1-chain DNAs in the cell.

FIG. 79 is an embodiment-illustrating diagram of the fluorescent light detection in the present invention and, in particular, of the fluorescent light  
15 detection that is effective in the DNA inspection. The same reference numeral as that in FIG. 71 denotes the same component. Instead of the multi-spot excitation lights illustrated in FIG. 71 the irradiation of which is performed with the spacing of 10 pixels, sheet-  
20 shaped excitation lights 2000 are employed. As is the case with the optical system in FIG. 70, fluorescent lights obtained by the irradiation with the excitation lights are separated from the excitation lights, then being detected by the 1-dimensional photomultiplier  
25 tube array or the 1-dimensional ultra-high sensitivity censor. The excitations by the sheet-shaped excitation beams are executed simultaneously over mutually adjacent, for example, 50 pixels in a 1-dimensional

direction, then being detected simultaneously by the 1-dimensional high sensitivity sensor. The scanning by the amount of, for example, 500 pixels is performed in the x direction perpendicular to the sheet-shaped beams that is longer in the y direction, thereby obtaining fluorescent light images by the amount of 50×500 pixels. Next, the sheet-shaped beams are displaced by the amount of 50 pixels in the y direction, then executing the above-described one scanning. Repeating this operation 10 times finally obtains the fluorescent light picture of 500×500 pixels. In the present embodiment, the background noise becomes slightly larger in comparison with the above-described embodiments where there is performed the irradiation with the isolated spot light or the spot lights separated with each other with the spacing therebetween. Nevertheless, in comparison with the cases where the irradiation is performed largely in the 2-dimensional plane-like manner, it is possible to execute the fluorescent light detection with a less noise. The signals obtained from the 1-dimensional photomultiplier tube array or the 1-dimensional ultra-high sensitivity sensor are weak lights, and accordingly the photon counting is executed.

FIG. 80 illustrates an embodiment for indicating a method of generating the multi-spot excitation lights used for the fluorescent light detection or the DNA inspection using the fluorescent

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light detection. In a laser 27g, there are provided windows at Brewster angle at both ends of a laser tube 271 filled with a laser medium. Laser lights emitted out therefrom are returned back by 100% reflection mirrors 272, 273. The energy of the laser light that is reciprocating between the resonator mirrors is usually more than 10 times as high as the beam energy extracted out of the laser resonator through the emitting window from the optical energy within the resonator. In the case of inserting a multi-spot or sheet-shaped beam generating hologram plate 270 into the laser resonator, since a hologram 2701 has been formed in advance so that the multi spots or the sheet-shaped beams are generated, as illustrated in the drawing, multi spots 2702 are reproduced through a lens 274. Incidentally, the laser light with which the hologram has been irradiated forms the multi-spot images at a diffraction efficiency of substantially 10%. The remaining light passes through the hologram without being varied as a 0th light, then reciprocating within the laser resonator. These multi spots are completely identical to the multi-spot lights at the time of immediately after having passed through the mask-like circular aperture array explained in FIG. 70. This condition makes it possible to irradiate a detection target with the multi spots with the use of the lens 24g in accordance with the configuration in FIG. 70. As a result, it is possible to obtain the multi-



spot lights or the sheet-shaped beams the intensities of which are almost 10 times higher as compared with those in the conventional case where the hologram is located in a laser-emitting optical path. Consequently, it becomes possible to execute the high-speed and high-sensitivity fluorescent light detection and DNA inspection.

In the above-described embodiment, the explanation has been given concerning the case of the excitation lights having 1 wavelength. The present invention, however, also exhibits the effects in the case of using the excitation lights having 2 wavelengths or 3 or more wavelengths. In this case, conventionally, when trying to execute the detection using the plurality of wavelengths, it has taken an exceedingly long time to do so. On the other hand, the execution of the photon counting detection using the multi-spot lights according to the present invention makes it possible to execute the detection with a high-sensitivity and at a high-speed.

1. A DNA inspecting method of irradiating a to-be-inspected DNA chip with excitation lights having a desired wavelength and of analyzing obtained fluorescent lights, said to-be-inspected DNA chip obtained by hybridizing a target with a DNA chip, said target obtained by adding a desired fluorescent material to a DNA fragment formed by a preprocessing from a DNA that is a detection target, said DNA chip including a plurality of L cells that are microscopic areas where a plurality of types of desired fragments are arranged in accordance with a predetermined regular rule, comprising the steps of:

guiding said obtained fluorescent lights to a  
fluorescent light detecting optical path,

separating and detecting said fluorescent lights from respective multi-spot lights generated by said multi-spot excitation lights onto said DNA chip, and

executing an inspection of said to-be-inspected DNA chip from positions and intensities of









in such a manner that a desired area on said DNA chip is irradiated with said multi-spot lights, and

a control system for determining and inspecting DNA information about said to-be-inspected DNA chip from fluorescent light intensities and fluorescent light positions of said desired area on said DNA chip, said fluorescent light intensities and said fluorescent light positions being detected by said driving means and said fluorescent light detecting means.

13. The DNA inspecting apparatus as claimed in Claim 12, wherein said multi-spot excitation light-generating optical system generates said plurality of M excitation light spots simultaneously, said plurality of M excitation light spots being arranged in a 1-dimensional or 2-dimensional manner with a fixed pitch on a straight line.

14. The DNA inspecting apparatus as claimed in Claim 12, wherein said control system arranges said plurality of M irradiation spots onto said DNA chip on a straight line with a spacing of substantially  $kd$  with reference to said spot diameter  $d$  and an integer  $k$ , and repeats an operation in sequence  $k$  times, said operation being an operation where, after said irradiation with said spot array has been performed during said time  $\Delta t$ , said array is displaced in a direction of said array by substantially  $d$  and said irradiation is performed again during said time  $\Delta t$ , and thereby executes said inspection toward  $kM$  spot positions in

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22. A DNA inspecting method of irradiating a sample with excitation lights so as to detect fluorescent lights, said sample being obtained by coupling a fluorescent molecule-added DNA fragment with a DNA corresponding thereto, comprising the steps of:

separating said fluorescent lights from said excitation lights, said fluorescent lights being emitted from respective multi spots obtained by irradiating said sample with said multi-spot excitation lights including a large number of M microscopic spots,

detecting said fluorescent light images emitted from said sample with the use of a plurality of weak light detecting devices capable of executing a photon counting,

photon-counting, individually, each of photon signals obtained from said respective detecting devices,

storing, individually, photon-counted numbers Npm detected by said respective detecting devices,

changing positions of said multi-spot lights and a position of said sample relatively so as to store in sequence said photon-counted numbers from said respective detectors,

collecting and storing data on said photon-counted numbers over a desired range on said sample, and

constructing a fluorescent light picture from said collected data so as to execute said DNA inspec-

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tion.

23. A DNA inspecting method of irradiating a sample with excitation lights so as to detect fluorescent lights, said sample being obtained by coupling a fluorescent molecule-added DNA fragment with a DNA corresponding thereto, comprising the steps of:

separating said fluorescent lights from said excitation lights, said fluorescent lights being emitted from irradiation areas having a long and narrow configuration, said irradiation areas being obtained by irradiating said sample with said sheet-shaped excitation lights,

detecting said fluorescent light images emitted from said sample with the use of a plurality of weak light detecting devices capable of executing a photon counting,

photon-counting, individually, each of photon signals obtained from said respective detecting devices,

storing, individually, photon-counted numbers Npm detected by said respective detecting devices,

changing positions of said irradiation areas and a position of said sample relatively so as to store in sequence said photon-counted numbers from said respective detectors, said irradiation areas resulting from said sheet-shaped excitation lights and having said long and narrow configuration,

collecting and storing data on said photon-

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constructing a fluorescent light picture from  
said collected data so as to execute said DNA inspec-  
tion.

26. The DNA inspecting method as claimed in Claim 22, wherein said multi spots are arranged on a 1-dimensional or 2-dimensional straight line.

28. A DNA inspecting method of irradiating a sample with excitation lights so as to detect fluorescent lights, said sample being obtained by coupling a fluorescent molecule-added DNA fragment with a DNA corresponding thereto, comprising the steps of:

separating said fluorescent lights from said excitation lights, said fluorescent lights being emitted from respective multi spots or sheet-shaped irradiation locations that is obtained by irradiating said sample with said multi-spot excitation lights or said sheet-shaped excitation lights, said multi-spot excitation lights including a large number of M micro-

scopic spots,

detecting said fluorescent light images emitted from said sample with the use of a plurality of M weak light detecting devices in an average pixel detecting time of (300  $\mu$ sec/M) or less,

storing, individually, signals obtained from said respective detecting devices,

changing, relatively, positions of said multi-spot lights or said sheet-shaped excitation lights and a position of said sample so as to store said signals in sequence,

collecting and storing said signals over a desired range on said sample, and

constructing a fluorescent light picture from said collected data so as to inspect said DNA.

29. A DNA inspecting method of irradiating a sample with excitation lights so as to detect fluorescent lights, said sample being obtained by coupling a fluorescent molecule-added DNA fragment with a DNA corresponding thereto, comprising the steps of:

separating said fluorescent lights from said excitation lights, said fluorescent lights being emitted from respective multi spots or sheet-shaped irradiation locations that is obtained by irradiating said sample with multi-spot excitation lights or sheet-shaped excitation lights, said multi-spot excitation lights including a large number of M microscopic spots the diameter or the focus-achieving width of which is

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smaller than 3  $\mu\text{m}$  and larger than 0.3  $\mu\text{m}$ , said sheet-shaped excitation lights having a width that is smaller than 3  $\mu\text{m}$  and larger than 0.3  $\mu\text{m}$ ,

detecting said fluorescent light images emitted from said sample with use of a plurality of weak light detecting devices,

storing, individually, signals obtained from said respective detecting devices,

changing, relatively, positions of said multi-spot lights or said sheet-shaped excitation lights and a position of said sample so as to store said signals in sequence,

collecting and storing said signals over a desired range on said sample, and

constructing a fluorescent light picture from said collected data so as to inspect said DNA.

30. A DNA inspecting apparatus, comprising:

an excitation light-source,

a mechanism for holding a sample obtained by coupling a fluorescent molecule-added DNA fragment with a DNA corresponding thereto,

a multi-spot excitation lights-generating optical system for causing a light to be simultaneously generated on said sample as a plurality of M multi-spot excitation lights having a spot diameter d, said light being emitted from said light-source,

an objective lens for causing said fluorescent material to be simultaneously irradiated with

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means for storing fluorescent light-detected information and fluorescent light-detected position information of said desired area on said sample that

have been detected by said driving means and said  
fluorescent light detecting means, and

means for determining DNA information of said  
to-be-inspected sample from said fluorescent light-  
detected information and said fluorescent light-  
detected position information.

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ABSTRACT OF THE DISCLOSURE

A method of inspecting a DNA chip and an apparatus therefor that allow a picture to be reconstructed in the following steps: A plurality of irradiation spots are formed on a DNA probe array mounted on a stage. Then, the stage is displaced in X, Y directions so as to execute a scanning, thereby irradiating substantially all the entire surface of the DNA probe array. Next, a plurality of emitted fluorescent lights, which are generated from the plurality of irradiation spot portions on the DNA probe array, are converged and are then detected simultaneously by multi detectors. Finally, a data processing apparatus processes the detected signals, thereby reconstructing the picture.

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